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THE LABORATORY COLONIZATION OF *ANOPHELES DARLINGI**

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The utility of laboratory colonies for the study of mosquito biology scarcely needs to be emphasized. A striking illustration is the amount of information that has accumulated concerning *Anopheles quadrimaculatus* since the announcement by Boyd (1932) of its successful colonization and the description of technique published by Boyd, Cain and Mulrennan (1935). Many species of anophelines have been successfully colonized in various parts of the Old World; others have proved refractory, even though subject to intensive study by various workers. In the New World, the subject seems not to have received the attention it deserves, and the poverty of our information about the biological characteristics of the South American anophelines, in particular, is probably in part directly due to the lack of readily available laboratory material for study. *Anopheles albimanus* was successfully colonized by Rozeboom (1936) in Panama, but his seems to be the only published account of laboratory maintenance of any species of the *Nyssorhynchus* group. Several species of *Nyssorhynchus* are known to be important malaria vectors, while the status of others is unknown or doubtful. The group, from the taxonomic point of view, is difficult, and it will probably be impossible to determine the significance of various morphological variants without laboratory analysis of behavior, comparable to the studies carried out in Europe with the mosquitoes of the *Anopheles maculipennis* group. Biological studies of *Nyssorhynchus* species are thus clearly needed, and attempts at laboratory colonization would seem to be the logical first step in any such program.

Some eight species of *Nyssorhynchus* have so far been found in the vicinity of the laboratory at Villavicencio, Colombia. Preliminary experiments have indicated that three of these, *Anopheles argyritarsis*, *Anopheles strodei* and *Anopheles darlingi*, can be fairly readily colonized. Serious and continued attempts with one species (*Anopheles rangeli*) have so far consistently failed; a preliminary attempt with another species (*Anopheles pessôai*) also failed. Our staff at the Villavicencio

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laboratory hopes eventually to make detailed studies of the laboratory behavior of all the local anophelines, and to describe attempts at colonization that failed as well as those that succeeded. In the meanwhile a preliminary description of the colonization technique used with *A. darlingi* may be warranted by the importance of the species and the general poverty of information on the laboratory behavior of *Nyssorhynchus* other than *A. albimanus*.

METHOD OF COLONY ESTABLISHMENT

The colony was established with adults bred from larvae collected in a roadside ditch near Villavicencio. The larval population of this particular ditch was 90 per cent *darlingi* and 10 per cent *rangeli*. No attempt was made to separate the two species, and adults bred out in about this proportion were released in a "room-sized cage" (a screened section of a laboratory room 2 meters high and 1.5 meters square). We had previously tested a pure culture of *A. rangeli* in such a room, releasing over 2,000 adults bred from eggs; but no fertile ovipositions resulted. In the present case, 50 to 75 pupae were placed in the room daily, and the eggs of the next generation were first obtained in quantity on the 13th day of the experiment. We continued to add adults bred from wild larvae for two weeks more, until pupae of the first laboratory generation were available. The colony has since been maintained for three months with no addition of outside material. No material with *rangeli* characters has been found in the colony other than the original material. This contrasts with another attempt at colony establishment in which mixed *argyritarsis* and *strodei* were involved: in that instance the mixture of species persisted for months, until the mixed colony was finally purposely destroyed.

Success or failure in the establishment of mosquito colonies depends on the development of proper techniques for raising larvae, for inducing the adults to mate, for obtaining adequate adult longevity, for providing blood meals, and for inducing oviposition. Each of these points requires separate study, and techniques may have to be modified for each anopheline species. We are far from satisfied that we have as yet discovered the most satisfactory methods of handling *darlingi*, but an outline of the methods currently used may be of value, since they have the virtue of at least serving to maintain the colony.

The larvae are raised with essentially the same mud and bread crumbs technique that was found to be successful with *Anopheles atroparvus* in Europe (Bates, 1941a). We have not been able to grow *darlingi* in quantities in hay infusions, or in algal cultures, or in yeast cultures. The only local anophelines that we have raised in such media are *pseudopunctipennis* and *argyritarsis*—apparently two species whose larvae are not at all exacting in their requirements. Our early attempts to raise *darlingi* and *rangeli* with mud and bread crumbs failed, until we discovered that success is contingent upon finding the right mud and the right water. Our laboratory well water, like the laboratory well water in Albania, is very unsatisfactory for anophelines, for reasons that we do not at present understand. *Anopheles darlingi* larvae all die within three days in this water. Rain water collected in a cement tank was also relatively unsatisfactory, and as a routine we now use water taken from a small stream near the laboratory. Most of the soils near the laboratory

have a high clay content, and these proved to be unsatisfactory for "mud." Again we tested a variety of soils, including mud from various breeding places. The most satisfactory to date is a surface loam from one of our study areas. An analytical study of the properties of these various waters and soils would undoubtedly be very interesting, but so far we have had no opportunity to use other than trial and error methods of testing. Certainly our experience here parallels the finding in Europe that the success of the technique depends on the type of soil and the type of water used in preparing the culture medium.

There seems to be no need for special techniques for inducing laboratory mating of darlingi. We made a few tests with lights of different colors, and observed sexually excited males and mating under the lights, but no discrete swarm formation such as characterized the European *Anopheles superpictus* and *Anopheles labranchiae* (Bates, 1941b). Except for special experiments, the colony has been left with natural light entering through windows. Mating or sexual activity has not been observed without artificial light, even though we have spent a great deal of time in the cage under twilight conditions; it obviously occurs, however, since large numbers of fertile eggs are laid.

Adult survival under cage conditions seems to be good without any special provision for humidity or resting places. Mean room temperature in the laboratory is 25°C., and day humidities vary between 70 and 80 per cent relative humidity. We tried placing a box covered with wet black toweling in the room, but it was not used as a day resting place by the mosquitoes, so the practise was abandoned.

Blood meals have been provided by man, calf, and agouti. Darlingi in the cage seldom bite by day (though they are not as refractory in this respect as strodei), but they bite man readily and promptly in darkness at night. Since the maintenance of a large mosquito colony on personal blood becomes tedious, laboratory animals are used for this purpose as far as possible. We have had no success in inducing darlingi to feed on guinea pigs, even when the mosquitoes and animals have been confined together in small cages; they will, however, feed moderately well on agoutis (*Dasyprocta*). A calf makes an even more satisfactory host than man, and seems to be best for routine purposes. A study of host preference with this mosquito would obviously yield interesting results.

Oviposition is obtained by leaving on the floor of the room two large dark-blue enamel pans (25 x 40 cm.) containing rain water. Eggs and larvae are removed twice weekly. Since this seemed to be a satisfactory device for oviposition, no other methods have been tested. Anophelines are apt to be reluctant to lay eggs in white dishes (Bates, 1940), so it seems always best to start, at least, with oviposition pans with a dark background color. It is interesting that the eggs are always scattered, never occurring in adhering batches of the sort often found with European anophelines. This would suggest that oviposition always occurs on the wing, though it has not been observed.

One attempt was made to establish a darlingi colony in a small cage (50 cm. square, 1 m. high). Three hundred and twenty-eight adults were released in the cage. Mating was occasionally observed when a dim blue light was placed over the cage. Of 5 females dissected 15 days after the start of the experiment, 2 were found to be

fertilized. The mosquitoes would bite if an arm was thrust into the cage in darkness, but they refused to bite guinea pigs left in the cage overnight. On one occasion 160 eggs were found in a small enameled dish left in the cage; otherwise no eggs were laid. Apparently the maintenance of *darlingi* in such a small cage would require a special study of factors governing feeding, mating and oviposition.

SUMMARY

A laboratory colony of *Anopheles darlingi* has been established in a cage 2 meters high and 1.5 meters square. The mosquitoes feed readily on man or calf, mate without special provision of light, and oviposit in large dark enameled pans of rain water. The larvae are raised most successfully with the mud and bread crumbs technique. Difficulty was found in establishing the species in a smaller cage. *Anopheles strodei* and *A. argyritarsis* can be colonized with the method used for *A. darlingi*, but similar attempts with *A. rangeli* and *A. pessoai* have failed.

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LABORATORY COLONY OF *ANOPHELES DARLINGI*¹

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The first attempt of the Malaria Research Service of the Medical Department of British Guiana to establish a colony of *A. darlingi* in the laboratory began on August 6, 1944, starting with 24 adults bred out from eggs laid by wild mosquitoes. The equipment and general technique used were the same as those described in this paper. That experiment was only partly successful, as the colony died out by the 23rd of November, after reaching the 6th generation. The number of adult mosquitoes raised in each generation was probably too small; and inbreeding may also have been a factor in the failure.

A second attempt has been completely successful; and an *A. darlingi* colony has been flourishing uninterruptedly in our field laboratory for over two years. Between November 29, 1944, and the end of December 1946 it passed through 35 generations.

MATERIALS AND METHODS

Cages. Cylindrical cages 60 cm. high and 35 cm. in diameter have been used throughout. A cage consists of a cotton mosquito-gauze sleeve stretched over a 10-gauge wire frame; the bottom of the frame is made of galvanized sheeting. The mosquito netting is knotted or tied tautly above and below the frame. Access to the cage is obtained through a lateral sleeve, wide enough to allow the easy passage of an ordinary sized finger bowl.

Three such cages are sufficient to keep the colony going, each generation being kept separate from the others. The cages are suspended from a rack in a sheltered, not too brightly illuminated, corner of the laboratory, well isolated from the attack of ants and spiders. This type of cage has proved much more satisfactory than the usual wood, wire mesh and glass structures, not only because it is cheap and easy to make, but because it offers no shelter to ants and spiders in particular, as these can be detected and removed easily before they have time to do irreparable damage.

Water containers. White glazed china finger bowls (diameter 11 cm., depth 7 cm.) are provided for oviposition in the cages. These are only half filled with water, to prevent spilling when the bowls are introduced into or withdrawn from the cage through the sleeve. The eggs are allowed to hatch in these bowls, and the young larvae are transferred to white enamel basins (28 cm. in diameter and 8 cm. deep), where they complete their development and pupate. The pupae are collected and transferred to finger bowls, which are introduced into the cage of the corresponding generation, for the adults to emerge. Bowls and basins throughout development are exposed to normal indoor light. They receive no direct sunlight.

¹ These studies were carried out by the Malaria Research Service of the Medical Department of British Guiana with funds contributed by the Colonial Government, the International Health Division of The Rockefeller Foundation and the British Guiana Sugar Producers' Association.

Selection of Water. This is an important point. Clean, fresh water with a pH ranging from 6 to 7 gives the best results. Acid waters, whether colorless or vegetable-stained, are not suitable, but can be used if their reaction is properly adjusted. Rain water, if caught directly in enamel trays, or off slate roofs, gives good results; but in the field laboratory here we have been unable to rear larvae in water draining off a painted galvanized zinc roof or stored in vats made of Wallaba wood (*Epeura falcata*). We have had excellent results with the local artesian well water in spite of its high iron content.



FIG. 1. Malariological Field Laboratory at Sophia, British Guiana. Equipment used for rearing *A. darlingi* colony. Three cylindrical cages like the one shown in this illustration (measurements: 60 x 35 cm.) are required to keep successive generations separate from each other. The china bowls and enamel basins, in which development from ovum to pupa takes place, are also shown.

A colony established in November 1944 was still flourishing in January 1947, having passed through 35 generations in 25 months.

Food. The cages for adult mosquitoes are supplied with cotton wads soaked in cane sugar solution, which are changed daily. The females are given an opportunity to take a human blood meal every day; to provide this the technician in charge of the colony introduces his bare arm into each cage for one-half hour every morning. No attempt has been made to use animals for this purpose.

For larvae, dried brewer's yeast proved to be the best food; and this was the only food used for a number of generations, until the supply gave out. Bread crumbs, powdered crackers, dog-biscuits, "Pabulum," ground shrimps and several other preparations gave very poor results. When these were used larval mortality was

high, particularly in the 4th instar, in the pupal stage or at emergence. In some cases the water was rapidly fouled by such foods and the pH was affected.

The prospects for the colony seemed poor, when we tentatively tried out a poultry feed manufactured by the Quaker Oats Co., Chicago, U. S. A., under the trade name of "Full-O-Pep Laying Mash." Results were satisfactory and we have used this food ever since, plain or mixed with yeast. If used in proper amount, it has very little fouling effect; and in a basin containing an average batch of some 100 larvae, the water need not be changed more than once or at the most twice during the whole cycle of development.

TABLE 1

Average monthly temperature and relative humidity in laboratory

1945 MONTH	TEMPERATURE		RELATIVE HUMIDITY PER CENT				
	Average daily max.	Average daily min.	6 hours	9 hours	11 hours	13 hours	21 hours
January.....	85.0	73.5	89	72	73	70	83
February.....	85.0	73.0	87	79	75	75	78
March.....	85.7	73.3	84	75	70	70	82
April.....	86.5	73.7	90	75	72	71	85
May.....	86.3	70.8	91	83	76	77	87
June.....	86.1	73.1	95	85	83	80	87
July.....	86.8	73.0	94	83	80	73	86
August.....	88.7	73.0	93	79	73	70	83
September.....	88.8	73.8	92	77	70	66	85
October.....	87.6	71.4	91	77	72	70	85
November.....	86.0	70.5	91	79	73	73	87
December.....	84.7	72.3	91	83	77	79	87

MICROCLIMATIC CONDITIONS IN THE CAGES

The field laboratory is surrounded by irrigated cane and rice lands offering an ideal natural habitat for *A. darlingi*. Adults of this species can be captured by day, often in large numbers, resting in the houses of the immediate neighborhood. Though more abundant during and after the summer rains, they can be collected in the area at all seasons and without difficulty. Obviously, under such conditions no special precautions appear necessary in order to create, artificially, suitable climatic conditions in the laboratory. All we have done is to protect the cages from direct draughts and excessive light.

The maximum and minimum temperatures were taken daily in the laboratory, and dry and wet bulb readings were made regularly at 6, 9, 11, 13 and 21 hours. The average monthly temperature and relative humidity readings are summarized in Table 1. It will be noted that seasonal variation is very slight, and it can be assumed that, under less favorable or less stable climatic conditions, the temperature and humidity range in the insectarium would have to be regulated to conform approximately to average conditions in our laboratory. A temperature of 80 degrees F. and a relative humidity of 85 per cent may be regarded as optimal. The range of

water temperature in the bowls and basins showed no significant difference from that of the atmosphere.

BEHAVIOR OF *A. DARLINGI* IN CAPTIVITY

Each anopheline generation consists of several hundreds or thousands of individuals, and it is impossible to quote figures which would represent true averages, as regards the duration of each developmental phase, for every generation. We have limited our analysis to the study of certain data, based on the date of the first oviposition, the first hatching, the first pupation and the first adult emergence in each generation. From these, we have calculated the interval between the first adult emergence in the parent generation and the first oviposition, as well as the duration of the larval and pupal stages, of the total developmental cycle (ovum to adult) and of a complete generation (adult to adult). These data, summarized in Table 2, obviously are based on the study of only a few individuals for each generation, i.e. the first produced.

In Table 2 time is stated in days, as observations concerning the colony are taken regularly each morning, i.e. only once a day. This may cause an error of one whole day. The pupal stage, for instance, usually lasts 36 hours. In the table it appears consistently as 1 or 2 days. Our figures, calculated as they are, are not averages, so that it is not surprising to see considerable variations in some of the values. Extreme deviations are probably the result of accident and as such have little or no significance. On the contrary, the tendency of values to recur consistently within a fairly narrow range, generation after generation and without relation to season, is the point of main interest in our tabulation.

The first oviposition in a generation occurs usually 6 to 8 days after the emergence of the first adults in the parent generation. The extreme periods noted were 4 days in the 30th and 23 days in the 12th generation. The ovum stage lasts consistently 2 days; the larval stage ranges from 6 to 16 days, but in two-thirds of the cases it varied from 6 to 12 days. The pupal stage never exceeded 2 days. The complete ovum-to-adult cycle ranged from 10 to 19 days, but in two-thirds of the cases it was 10 to 14 days.

The minimum adult-to-adult period, i.e. the interval between two connective generations, was 16 days and the maximum 35 days. Considering that in the 25 months from November 29, 1944, to December 31, 1946, the colony produced 35 generations, the average interval between two successive generations, under our laboratory climatic conditions, can be calculated at 21.7 days. As an average, three generations are active in the colony at one time, so that three cages are found sufficient to keep the colony going throughout.

During the day, the adults remain at rest and do not fly unless disturbed; but if a bare arm is thrust into the cage many females will attack it and feed without hesitation at any time, even in full daylight. As a routine, the females are offered a blood meal between 8 and 10 each morning.

At dusk the mosquitoes become active and begin flying around the cage. In the narrow space allowed in our cages, though swarming and mating obviously occur, conditions are not quite favorable: 99 per cent of wild caught females are fertilized;

but among females of our colony the highest rate of fertilized individuals ever noted was 79 per cent.

Some experiments were made to determine the relation of fertilization to the age of the female and to blood meals. Batches of 50 males and 50 females were used in

TABLE 2
A. darlingi colony

GENERATION	INTERVAL BETWEEN 1ST ADULT EMERGENCE AND 1ST OVIPOSITION	DURATION OF OVUM STAGE	DURATION OF LARVAL STAGE	DURATION OF PUPAL STAGE	DURATION OF OVUM TO ADULT CYCLE	ADULT TO ADULT CYCLE	SEASON
		<i>days</i>	<i>days</i>	<i>days</i>	<i>days</i>	<i>days</i>	
I							Nov. 1944
II	15	2	7	2	11	26	Dec. 1944
III	7	2	8	1	11	18	Jan. 1945
IV	13	2	10	1	13	26	Jan.-Feb. 1945
V	7	2	8	2	12	19	Feb. 1945
VI	17	2	10	1	13	30	Mar. 1945
VII	6	2	6	2	14	20	Apr. 1945
VIII	6	2	6	2	10	16	Apr.-May 1945
IX	6	2	8	2	12	18	May 1945
X	5	2	9	2	13	18	May-June 1945
XI	8	2	8	2	12	20	June 1945
XII	23	2	9	1	12	35	July-Aug. 1945
XIII	8	1	14	2	17	25	Aug. 1945
XIV	8	2	12	1	15	23	Sept. 1945
XV	11	2	14	2	18	30	Oct. 1945
XVI	7	2	16	1	19	26	Oct.-Nov. 1945
XVII	10	2	12	1	15	25	Nov.-Dec. 1945
XVIII	8	2	9	1	12	20	Dec. 1945
XIX	7	2	14	1	17	24	Jan. 1946
XX	7	2	13	1	16	23	Feb. 1946
XXI	8	2	11	1	14	22	Feb.-Mar. 1946
XXII	6	2	11	1	14	20	Mar. 1946
XXIII	7	2	10	1	13	20	Apr. 1946
XXIV	9	2	8	1	11	20	Apr.-May 1946
XXV	8	2	10	1	13	21	May 1946
XXVI	8	2	13	1	16	24	June 1946
XXVII	6	2	14	1	17	23	June-July 1946
XXVIII	6	2	10	1	13	19	July 1946
XXIX	7	2	14	1	17	24	Aug. 1946
XXX	4	2	11	1	14	18	Aug.-Sept. 1946

cages of standard dimensions. In two experiments in which mosquitoes 24 to 36 hours old were used, with only sugar and raisins as food, only 2.27 per cent and 6.52 per cent, respectively, were fertilized 24 hours after introduction into the cage.

In a third experiment, the mosquitoes (of the same age) were left in the cage for 48 hours, but only 2.38 per cent were fertilized.

These experiments were repeated using only females which had had a blood meal;

the fertilization rate, after 24 hours was 10 per cent, and after 48 hours 20 and 27.3 per cent.

In a last experiment, the mosquitoes were left for 5 days in the cage, with only sugar, water and raisins as food; 46 per cent of the females were fertilized. It would appear that in our cages pairing is not easy and fertilization proceeds at a slow rate. It is not likely, as our findings might suggest, that in nature many females take a blood meal before pairing, otherwise the percentage of unfertilized females among blood-gorged individuals captured in houses would be certainly much higher than it is.

It would be interesting to investigate the relation of cage dimensions to fertilization rates.

The general technique required for the rearing of *A. darlingi* in captivity is so simple that this species might well be employed whenever large numbers of laboratory-bred anopheles are needed for experimental purposes, particularly if an efficient carrier of malaria or filariasis is required.

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SUMMARY

A laboratory colony of *A. darlingi* started in November 1944 has been successfully reared through 35 generations in 25 months. A description is given of the simple technique followed in this experiment. The behavior of this dangerous malaria vector in captivity is also discussed.

STUDIES ON IMPORTED MALARIAS: 3. LABORATORY REARING OF WESTERN ANOPHELINES*

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To determine experimentally the ability of western anophelines to become infected with and transmit foreign malaria relapsing in returning troops, a large and continuous production of anophelines was needed (Moore et al 1945). This report details methods used and experience gained in colonizing and producing large numbers of *Anopheles maculipennis freeborni* Aitken. Information gained concerning production of *A. maculipennis occidentalis* (D. & K.), *A. punctipennis* (Say), and *A. pseudopunctipennis franciscanus* (McCracken) is also reported. During the period from January to October, 1944, approximately 89,500 pupae of *A. m. freeborni*, 2,000 pupae of *A. m. occidentalis*, 2,000 pupae of *A. punctipennis*, and 500 pupae of *A. pseudopunctipennis franciscanus* were reared in the insectary located in the Letterman General Hospital, San Francisco, California.

For the purposes of this report, the nomenclature of Aitken (1945) has been followed for these anophelines.

METHODS

The following methods of rearing larvae and handling pupae and adults were found adequate for a fairly steady production of mosquitoes in the laboratory.

Insectary. The insectary was a room of 1,050 cubic feet, the walls and ceiling of which were painted dull white with a fungus resistant paint. Light was obtained from two 48-inch, 40-watt, double tube fluorescent fixtures and 21 x 31-inch north-facing window at ground level. A double curtained trap led to the door. Cages were covered with plastic screen and had cloth sleeves. Larvae were reared in rectangular enameled pans two inches deep and of various outside dimensions. Air temperature was maintained at 28°C. plus or minus 2°C. and the relative humidity between 80 and 95 per cent. Water temperature of rearing pans averaged 27°C.

Water Used in Raising Larvae. The tap water available was not found suitable. Although both protozoa and larvae would survive for a few days no larval growth was obtained. Water from a breeding source of *A. m. occidentalis* was found satisfactory for raising the four species of anopheline larvae. Not more than one batch of larvae could be reared in the same water without high mortality although the used water had a higher protozoal content than before use. Distilled water was added to pans to maintain the primary level of 1½ to 2 inches.

* Contribution from the Imported Malaria Studies program of the National Institute of Health and Malaria Control in War Areas, U. S. Public Health Service, Columbia, S. C.

Appreciation is expressed to the staff of the Letterman General Hospital who furnished quarters for the insectary and to the Division of Entomology, University of California, Berkeley, where some preliminary experiments were run.

Larval Food. Fresh pond water contained sufficient "food" for newly hatched larvae for two or three days as evidenced by their growth. After this time it was necessary to add supplementary food. Dehydrated, finely ground dog food of various sorts were tried as supplementary food. A mixture of dog food of about 20 per cent protein and 10 per cent dry brewer's yeast was found satisfactory.

After grinding and mixing, the food was sifted through suitable material to exclude the larger particles. Food was dusted on the surface of water when the surface became apparently free of bacterial "scum" or of food particles. Differential hatchability of each batch of eggs and mortality of larvae from one pan to another necessitated treating each pan of larvae as a separate problem as to feeding frequency and amount fed.

Technique of Handling Eggs, Larvae, and Pupae. A heavy, gelatinous "scum" formed on the pond water surface within a few hours after the water was poured into breeding pans. Because this "scum" trapped and killed newly hatched larvae it was found necessary to incubate eggs 2 days in a small bowl or half-pint carton and then rinse the eggs into pans of fresh pond water. In this way the small larvae consumed the "scum" as it formed without becoming trapped. It was found that approximately 200 larvae per square foot of water surface could be reared through the second instar. However, after the second instar 200 larvae per square foot would consume food so rapidly that overnight they exhausted all food particles that could be loaded on the water surface and then consumed the setae on one another. When conditions permitted larvae were given more surface area.

A large flagellated rod bacterium was frequently found at the water surface in larval pans associated with very high mortality of larvae. Dissections of larvae obviously affected by something in these "cultures" showed this bacterium packed in the alimentary canal and alive as evidenced by their motility when expressed and examined. Larvae which had recently died were found to have large numbers of this bacterium in their body tissues. To reduce this hazard, pipettes used for removing pupae and handling larvae were rinsed with alcohol between usage in order to prevent contamination from one pan to another.

Pupae were removed once daily to half-pint cardboard cartons and rinsed with clear water to free them of larval "culture." If pupae were not rinsed a heavy gelatinous "scum" formed in which large numbers of adults became trapped during emergence resulting in some instances in 100 per cent mortality. After rinsing two or three large larvae were put in each carton to consume any "scum" that formed.

RESULTS

With the above techniques and under the pressure of trying to obtain as many pupae as possible with the space limitations, it was noted that in the first and subsequent generations the body size of larvae, pupae, and adults was obviously smaller than that of field-collected specimens. This was true with all species reared from eggs as well as with *A. m. occidentalis* reared from field-collected first, second, and third instar larvae. A few experiments in which 10 *A. m. freeborni* larvae were reared per square foot instead of 100 to 200 showed that given sufficient water

surface area and reduced competition for food, larvae, pupae, and adults were produced that were approximately the same size as those collected in the field.

During a period of 92 consecutive days 39,750 *A. m. freeborni* pupae were obtained from approximately 74 square feet of water surface. The time interval from egg laying until the formation of the last pupae was about 21 days at 27°C. (Table 1).

A check of samples of *A. m. freeborni* pupae totalling 21,389 revealed that 4 per cent failed to emerge successfully.

A. m. freeborni. Progeny of field-collected females from Riverside, Merced, and Auburn, California, were reared and mated successfully in 10, 12, and 20-inch cubed cages during a period from January to March. No gross differences were found

TABLE 1

Comparison of Different Generations of A. m. freeborni at 27°C. as to Rate of Development and Variations

GENERATIONS REMOVED FROM FIELD-CAUGHT FEMALES	NO. LOTS* EXAM- INED EACH GENER- ATION	TOTAL NO. PUPAE	DAY OF 1ST PUPAE		DAY ½ WERE PUPAE		DAY ¾ WERE PUPAE		DAY ALL WERE PUPAE	
			Av.	Range	Av.	Range	Av.	Range	Av.	Range
Eggs from Field Females...	25	5,114	13.1	11-19	16.7	12-24	18.1	14-26	20.6	15-28
1st.....	30	6,127	14.8	11-21	18.8	13-24	20.3	15-16	22.4	15-28
2nd.....	31	3,679	13.2	10-18	15.3	10-22	16.2	11-24	18.9	12-30
3rd.....	31	5,586	13.2	11-17	15.6	11-22	16.7	11-22	18.6	13-26
4th.....	26	3,445	13.4	11-17	15.2	11-21	16.3	13-19	17.9	14-25
5th.....	16	1,914	15.4	11-19	20.3	13-27	22.2	15-28	25.6	15-28
6th.....	28	3,024	14.5	12-19	18.2	15-22	19.7	16-24	22.3	18-26
Total of 6 Generations...	162	23,775		10-21		10-27		11-28		12-30
Average of 6 Genera- tions.....			14.1		17.2		18.6		20.95	

* In this table and subsequently, a lot is defined as a quantity of eggs put in a single pan. The quantity was varied with the size of the pan.

between the groups from these different areas. Because of the relative abundance of this species at Marysville, California, females were collected there and were used to obtain a permanent colony. This colony was continued from March to October without the necessity of adding new stock from the field. During this time seven generations were completed. No attempt was made to obtain as many generations as possible in this period.

To obtain mating and eggs, 500 to 1,000 pupae were introduced into plastic-screened cages 20 inches cubed. Adults that emerged (from 1 to 3 days after being collected) were given freshly cut surface of apples each evening until spermathecal examination of a sample of females showed over one-half inseminated. Without apple or some other source of sugars no insemination occurred. Usually 10 to

15 days contact with males was necessary before one half of the females were inseminated. In a few batches after 10 days of contact with males, all females dissected were found inseminated. No relationship could be found between blood feeding of females and insemination or between insemination and ovarian development. After females were inseminated they were fed on man and/or rabbit. By using four mating cages and staggering the introduction of pupae a fairly steady production of eggs resulted.

A pad of cloth large enough to cover the top of the mating cage and saturated with 5 per cent glucose solution was found superior to apple feeding in the resultant number of females producing eggs. It was found necessary to wash the pad thoroughly each day to prevent the development of yeast.

The production of eggs was increased by placing 10 to 15 inseminated females over water in small carton cages and by giving them an opportunity to feed on man every day. By this method, about 20 per cent of the total pupal production was necessary for the number of eggs needed.

In a single experiment, 20 non-blood-fed females selected at random from mating cages were placed in half-pint carton cages covered on both ends with bobbinet. A pad of cotton was saturated with either water or 5 per cent glucose in water and placed on top of the bobbinet. The cages were put in a refrigerator held at 4°C. and the pads renewed or resaturated when necessary. It was found that when water only was available one half were dead in 64 days (average length of life 40 days) whereas if 5 per cent glucose solution was available one half were not dead until 124 days (average length of life 64 days). Apparently, the females were able to move and imbibe the glucose solution and water at this temperature (?) as their crops were frequently found distended with a clear liquid. Perhaps they moved and fed when the temperature was elevated temporarily when the door was opened. Upon the basis of this experiment, continuous egg production was obtained by routinely refrigerated females.

Refrigeration at 4°C. of freshly laid eggs was not found practical beyond 10 days as after this time the number hatching fell rapidly to zero.

The difference in hatchability of eggs from field-collected females compared with the eggs from successive generations of laboratory-reared females (Table 2) is difficult to understand considering the finding that extremely few females laid eggs when not inseminated even though replete with them.

A comparison of survival to the pupal instar (Table 3) and rate (Table 1) of development of laboratory-reared *A. m. freeborni* showed no definite relationship with the generation. True relationships may be masked by the relatively low, 58 per cent, (Table 2) survival of larvae.

Since the gross size of laboratory-reared adults and the hatchability of their eggs were different from the field-collected females, it was considered essential to compare field-collected females and laboratory-reared females in other respects.

Table 4 summarizes the results of two experiments carried on at 28°C. comparing laboratory-reared females randomly selected with randomly collected females (near Marysville, California). The first collection in April of field females is compared in Table 4 with second generation females from the laboratory; the second collection in

September divided into two groups is compared with the fourth generation from the laboratory. All females were confined separately in carton cages over water and given an opportunity to feed on man at least every other day. Some of the field-

TABLE 2
Hatchability of Eggs of Various Generations of A. m. freeborni at 27°C.

GENERATIONS REMOVED FROM FIELD FEMALES	LOTS CHECKED	EGGS COUNTED	LARVAE HATCHED	PER CENT HATCHED	SURVIVAL OF THOSE HATCHING		
					Sample Size	Number Pupae	Per Cent Pupated
By field females.....	1+	2,500*	2,000*	80†			
2nd.....	16	6,695	3,278	49.0	3,278	1,772	53.9
3rd.....	109	46,078	24,375	52.9	21,494	13,547	63.0
4th.....	15	5,603	3,492	62.3	3,015	1,505	49.9
5th.....	20	8,615	4,632	53.8	4,632	1,915	41.3
Total of 2, 3, 4, and 5th generations.....	160	66,991	35,777	53.4	32,419	18,739	57.8

+—One night's laying of many randomly collected field females.

* Estimated.

† Approximately.

Survival data were selected to exclude information from those pans infected with the deleterious bacterium, described above.

TABLE 3
Comparison of Survival of A. m. freeborni of Different Generations from Egg to Pupae at 27°C.

GENERATIONS REMOVED FROM FIELD FEMALES	NUMBER LOTS	NUMBER EGGS	NUMBER PUPAE	PER CENT PUPATING
By field females.....	25	11,843	5,114	43.2
1st.....	30	12,452	6,127	49.2
2nd.....	31	9,989	3,679	36.8
3rd.....	31	10,780	5,586	51.8
4th.....	26	10,356	3,445	33.2
5th.....	16	6,131	1,914	31.2
6th.....	28	7,915	3,024	38.2
Total for 6 Generations.....	162	57,623	23,775	41.3

Data are from females collected separately and colonized separately from those reported in Table 2.

Data were selected to exclude those lots where the water was found infected with the deleterious bacterium described above.

collected females must have had at least one blood meal previously whereas laboratory-reared females had no blood before being placed in the cages. Although the samples studied were admittedly small they were nevertheless chosen at random.

It is interesting that although the previous history of the field females as to age and nutrition was not known, they lived about as long after being transported in a cage 120 miles as reared females and laid on the average considerably more eggs per laying and per life than did the laboratory-reared females.

The maximum number of eggs deposited by one field-collected female *A. m. freeborni* was 1,527 and the average for 34 females was 751 eggs in their lifetime, or 176 per laying. Two females at one oviposition laid 432 and 408 respectively (Table 4). These results may be compared with the findings of Herms and Freeborn

TABLE 4

Comparison of Field-Collected with 2nd and 4th Generation Laboratory-Reared A. m. freeborni at 28°C. as to Longevity, Egg Production, and Number of Blood Engorgements

MATERIAL AND CONDITIONS	NUMBER FE- MALES STUDIED	DAY $\frac{1}{2}$ DEAD	MAX. LENGTH LIFE DAYS	AV. LENGTH LIFE DAYS	AV. NO. EGGS PER FEMALE PER LAYING	AV. NO. EGGS PER FEMALE	GREATEST NO. EGGS		ENGORGEMENTS	
							By one Fe- male	At one Time	Av. Per Fe- male	Greatest No. by one Fe- male
Field, April Water & Blood	18	App. 27	41	25.0	194	798	1,527	432	7.5	20
Field, Sept. Water & Blood	8	27	48	25.0	192	768	1,388	409	5.5	10
Field, Sept. 5% Glucose & Blood	8	21	40	29.8	148	686	1,129	308	5.9	14
Averages.....	34	25	44.2	26.6	176	751			6.3	
2nd Generation Water & Blood										
Laid eggs	8	24	36	28.0	101	339	734	183	7.0	12
Laid no eggs	11	18	31	19.0					4.8	9
4th Generation Water & Blood										
Laid eggs	11	29	36	27.7	130	440	707	279	5.7	8
Laid no eggs	8	23	29	23.0					4.8	6
Averages of Those Lay- ing Eggs.....	19	26.5	36	27.8	115.5	389.5			6.3	
Averages of Those Not Laying Eggs.....	19	20.5	30	21.0					4.8	

(1920) who report a maximum of 315 eggs laid by a field-collected female and for 30 females—an average of 209 eggs. In subsequent work Herms and Frost (1932) report a maximum of 288 and an average of 195. Aitken (1945) reports a maximum of 268 for one laying and an average of 106 from females collected at Sunol and in the Sacramento and San Joaquin Valleys. He also found a maximum of 149 and an average of 95 for females collected at Point Reyes station, Marin County, Valley Ford, Sonoma County, and Castroville, Monterey County.

A. m. occidentalis. Two attempts at colonization of the species failed in 1943 and 1944 with progeny of field-collected females reared in the laboratory. Females were

collected at Hamilton Field, Palo Alto and an area 13 miles south of San Francisco in irrigation reservoirs occurring near the ocean. Cages tried were 20 inches cubed, 54 x 28 x 28 inches, and 5 x 5 x 12 feet. The first two cages were tried in the insectary and the last one at the University of California Laboratory of Insect Physiology. Although both sexes fed readily on fruits, honey and sugar solutions (as does *A. m. freeborni*) and the females fed readily on man, only one batch of eggs was laid, none of which hatched. Dissection of a few of these eggs showed no embryos.

On one occasion, 12 females were collected in the field but only 6 laid eggs when confined singly in carton cages. The greatest number of eggs laid by one female was 894 during its life in the insectary when fed blood at least every other day. The greatest number of eggs laid at one time by one female was 333. The average per laying was 202 and the average per female during life under caged conditions was 337.

Examination of a quantity of freshly emerged progeny of positively identified field-collected *A. m. occidentalis* revealed no intergradations of the pale wing tache with the unicolorous condition found in *A. m. freeborni*.

A. punctipennis. Three attempts to colonize *A. punctipennis* from Auburn and Hamilton Field, California, failed. No particular difficulty was experienced in rearing progeny of field-collected females, but attempts to satisfy the conditions for mating failed. Adults fed readily on fruit and 5 per cent glucose solution and females fed on blood repeatedly but laid no eggs although their ovaries were mature.

A. pseudopunctipennis franciscanus. Two of three attempts with this species to obtain mating in cages were successful. Females for oviposition were collected in Marysville and Palo Alto, California. The size of cage seemed to have some influence as the smallest cage in which mating was successful was 14 x 14 x 36 inches. It was found necessary to blood-feed females at least once before insemination occurred. Furthermore, no females were found inseminated until all traces of the previous blood meal were gone from the mid gut. This is in contrast to *A. m. freeborni* where females mated without reference to blood feeding. A small colony was carried through two generations without any special difficulties except in inducing females to feed on man or rabbit. Many females would die of starvation before they would feed on man or the rabbit. However, they were highly attracted to a cow and would engorge rapidly when applied. This tends to substantiate Reeves' (1944) tentative conclusion that "cow appeared to be its preferred host." The colony was stopped as no further use was found for specimens.

A number of eggs would frequently sink to the bottom of the water in which they were laid. These sunken eggs would hatch but required from 2 to 4 days longer than the ones that remained at the surface. Both *boydi* and *franciscanus* types of eggs figured by Aitken (1945) were produced from a single first generation laboratory-reared female. Some of these eggs exhibited a form taken to be intermediate between the two types.

SUMMARY AND CONCLUSIONS

1. A colony of *Anopheles maculipennis freeborni* was maintained for a 7-month period during which time approximately 89,500 pupae were obtained and 7 generations were passed through.

2. *A. m. freeborni* mated readily in cages as small as 10 inches cubed if utilizable carbohydrates were available.
3. Water from a natural breeding site of *A. m. occidentalis* was found suitable for rearing larvae of all species whereas the tap water available was not suitable.
4. A supplementary food composed of 10 per cent dry brewer's yeast and 90 per cent finely ground dehydrated dog food with a total protein content of about 20 per cent was found adequate for feeding larvae.
5. An average of approximately 122 larvae of *A. m. freeborni* were reared per square foot of water surface $1\frac{1}{2}$ to 2 inches deep. A batch of larvae required on an average of about 21 days from oviposition to the pupation of the last larvae. The range was from 12 to 30 days at an average water temperature of 27°C.
6. *A. m. freeborni* survival from first instar to pupae under insectary conditions was approximately 58 per cent.
7. Pupal mortality was reduced from a high percentage to approximately 4 per cent when the pupae were washed free of larval "culture" and large larvae were added to reduce bacterial "scum."
8. No consistent difference was found in the rate of development between laboratory-reared progeny of field-collected *A. m. freeborni* and successive generations.
9. Laboratory-reared *A. m. freeborni* were not equivalent to field-collected adults as to gross body size, when 100 to 200 were reared per square foot of surface area. At 10 per square foot, the size was about the same as field-collected specimens. The average number of eggs laid per lifetime per female and the average hatchability of eggs laid were less in the laboratory-reared than in the field-collected females.
10. Two successive generations of *A. pseudopunctipennis franciscanus* were obtained without difficulty when the cage was 14 x 14 x 36 inches or larger and when females were fed at least once upon blood.
11. No evidence was obtained of *A. m. occidentalis* or *A. punctipennis* mating under conditions suitable for *A. m. freeborni*.

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PRACTICAL LABORATORY METHODS FOR QUANTITY REARING AND HANDLING OF *AEDES AEGYPTI* MOSQUITOES TO BE INFECTED WITH *PLASMODIUM GALLINACEUM*

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The methods of rearing and handling large numbers of *Aedes aegypti* mosquitoes herein described have been developed over a period of two years in connection with studies of *Plasmodium gallinaceum* in chickens. The paper is divided into two parts—the rearing of the insects, and the handling of the adults when infected with *Plasmodium gallinaceum*.

PART 1—REARING OF THE INSECTS

The quantity rearing of *Aedes aegypti* in the laboratory involves the care of the colony of adults, the gathering, care and hatching of the eggs, the feeding of the larvae, the gathering of the pupae, and the handling of the adult insects prior to feeding on chickens. All of these operations are carried on in a single room, screened in accordance with Department of Agriculture recommendations and maintained at an average temperature of $75 \pm 3^{\circ}\text{F}$. and a relative humidity of 70 to 75 per cent. Each of the operations will be described separately.

Adults. The colony cages consist of individual cubical wooden frames, approximately three feet in each dimension. The cages have 18 mesh wire screened sides. The front of each cage is of glass or plastic material, with the exception of a 16-inch square opening near the bottom. This opening is enclosed by the usual type of cloth sleeve, through which the caged insects are serviced.

The adult colonies of insects were established from larvae taken in nature from artificial containers near the laboratory.

In order that the adult insects may obtain a blood meal and produce fertile eggs, a rabbit with shaved sides is provided. The rabbit is held in a closely fitting, yet not cramping cage. This cage is a modification of the type used at the Savannah Laboratory of the U. S. Public Health Service, and consists of chicken wire sides and top on wooden ends. An easily detachable bottom facilitates rapid handling of the rabbit. The rabbit, enclosed in its small cage, is introduced into the colony cage for approximately two hours each morning. Only a part of the female mosquitoes take a blood meal each day, but the egg production is entirely adequate. Figure 1 shows the rabbit in its cage.

A cotton wick in a wide-mouth 4- or 6-ounce bottle, kept moist with a 5 per cent sugar water solution, provides food for both male and female insects and is kept in each colony cage.

Wet cellulose sponges, about 3 x 4 x 1 inches, in open shallow enamelled pans are provided in each cage, upon which the insects deposit eggs. It has been found

advantageous to keep a small depth of water in the pans to prevent drying out of the sponges.

Eggs. Every other day the wet egg-containing sponges are removed from the colony cages and replaced by others. Johnson (1937) confirmed that a "conditioning" period for the eggs is necessary if they are to hatch promptly and completely when desired. This "conditioning" consists in keeping the sponges wet in glass dishes for a period of at least 48 to 72 hours before allowing them to dry. At the end of the 48 to 72 hour period, the sponges are gently pressed to remove surplus water and allowed to dry. The conditioning and drying takes place at the usual insectary temperature and humidity. The dried egg-containing sponges can be stored at ordinary temperature up to at least two months, and the eggs will promptly hatch when the sponge is immersed in water (Shannon and Putnam 1934). This storage period enables a reserve of eggs to be built up to meet any unusual demands.

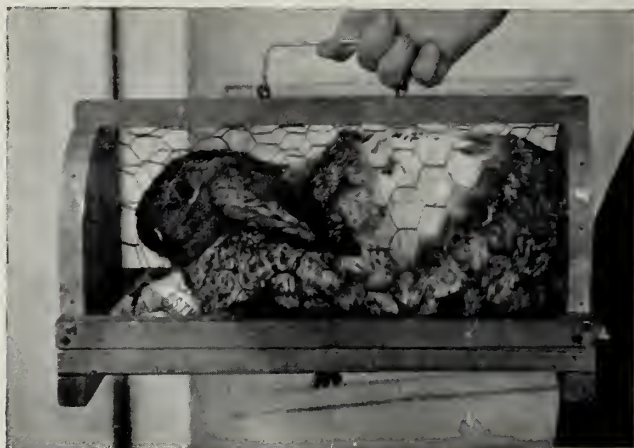


FIG. 1. A RABBIT IN A SMALL CAGE PROVIDES BLOOD MEAL FOR MOSQUITOES

At this laboratory a two-quart glass beaker has proved satisfactory for holding the hatching egg sponges. No food has been found necessary at this stage.

Larvae. The hatch of eggs is complete in 24 to 36 hours, and the small larvae are then removed to rearing pans. White enamelled round pans, about 15 inches in diameter and 4 inches deep, are generally used, although any similar pan, round or square, is suitable. The pans are three-fourths filled with tap water, and from 1200 to 2400 larvae (estimated) are placed in each pan. The larvae are fed once a day. Pablum ground to a powder is used as larval food.

From 300 to 600 pupae develop in each pan in from 6 to 10 days.

The heavy feeding causes the water in the pans to become foul and odorous, but unless a heavy white tenacious scum is found, no damage to the growing larvae results. It is customary to feed the larvae in each pan a heaping tablespoon of Pablum each day, and to drain off and replace a part of the water daily.

The larvae of *Aedes aegypti* are light sensitive and will readily congregate in a

clump at the opposite side of a pan from a strong light. This fact can be made of use in draining the pans without losing the larvae.

Pupae. Where the feeding of the larvae has been generous, the female pupae can readily be distinguished from the males by their larger and bulkier size.

As the pupal stage lasts about two days, the pupae are picked from the pans daily. At this laboratory 800 female pupae per day are picked for experimental feeding on chickens. These pupae are picked with a wide mouth medicine dropper and placed in crockery sugar bowls in fresh water at a rate of 100 female pupae per bowl. Ordinary lantern chimneys with bobbinet covered tops are placed on the sugar bowls; the date is written on the chimney and the unit set aside so that the adult insects will emerge.

Cotton sponges wet with tap water are kept on the bobbinet chimney tops until the night before the adult insects are to be fed on chickens (wet cotton is removed on the night of third day after the date on the chimney).

After the 800 female pupae are gathered, the remaining pupae in the pans (male and female) are picked with a wide mouth medicine dropper, on which a suction is maintained by a water faucet suction pump through a filtering flask. This is a very rapid method of picking pupae, and all the pupae can be collected in the filtering flask in a matter of not over ten minutes per pan.

The mixed pupae are transferred in glass ice box dishes of clean water to the colony cages to replenish the colony. A screened enclosure is kept over the pupae-containing dishes (raised daily to liberate adults) to prevent deposition of eggs.

It has been found that, with disposition of pupae as outlined, the colonies always contain a suitable number of male and female insects.

Handling of Adults Preliminary to Feeding on Chickens

The female pupae in the chimney-covered sugar bowls will produce adults in 1 to 3 days. The routine at this insectary is to remove the wet cotton at the close of the third day following the date of collection. On the morning of the fourth day after the chimney date, two pasteboard slides are inserted between the bowl and chimney, and the chimneys containing adult insects are delivered to the animal feeding portion of the insectary. Any insects remaining in the bowls and held there by the lower cardboard slide are destroyed.

While only 50 to 75 per cent of the pupae originally placed in the bowls appear as adult insects in the chimneys, it has been found that if a filter paper with a 1-inch diameter hole is kept between the bowl and chimney, a fewer number of the insects will become entangled in the water in the bowl. Recent use of these filter paper disks indicates that up to 80 adults from 100 pupae can readily be caged in the chimneys.

Operating Requirements of this Insectary

The insectary, operated as described, has been producing an average of 2000 pupae per day for the past 18 months. Of the 2000 pupae produced daily, 800 female pupae have been diverted daily for experimental infections with *Plasmodium gallinaceum*. The remainder are normally used to replenish the colony.

If it is assumed that chimneys containing mosquitoes will be kept three weeks, the following equipment is necessary to maintain our production: 2 colony cages, 20 larvae rearing pans, a supply of glass ice box dishes for handling sponges, pupae, etc., 200 gauze top lantern chimneys, 200 crockery sugar bowls to fit the chimneys, a supply of sponges, gauze and cotton, necessary larvae and pupae picking tubes, 3 rabbits and 2 small cages for confining the rabbits, and a supply of ground Pabulum.

With the above equipment and a convenient water supply, one full-time female employee and a half-time assistant readily handles the work required for the production of 2000 pupae per day.

PART 2—HANDLING OF FEMALE *Aedes aegypti* ADULTS FOR INFECTION WITH *PLASMODIUM GALLINACEUM*

Preparation for Feeding on Infected Chickens

The female *Aedes aegypti* are delivered to the chicken feeding portion of the insectary in lantern chimneys, as described in Part 1. These chimneys contain the hatch from the 100 pupae and usually result in 50 to 75 usable adults per chimney. The chimneys have bobbinet covered tops and simply rest on cardboard bottoms.

The adult mosquitoes are allowed to bite chickens beginning about noon, and as the moist cotton has been removed the previous night, the insects are presumably hungry.

For holding the chimneys during feeding, small platforms are provided, extending out in front of a table or long shelf. The platforms used in this insectary are slightly larger than the chimney bottom; are provided with metal tops, and on each side integral metal strips extend up nearly to the top of the chimney. The ends of the strips are turned down to form hooks. The chimneys are held on the platform by heavy rubber bands stretched over the chimney tops and caught under the hooks. A one-inch hole is provided in the platform, and is closed with two thicknesses of rubber dam provided with cross slits. Through this opening, the insects can be removed with a suction tube.

The methods used for the handling of adult mosquitoes are essentially the same as those employed in the mosquito insectary of the Division of Physiology, National Institute of Health, Bethesda, Maryland, and described by Trembley (1944).

Feeding of Mosquitoes on Chickens

The procedures described for holding chickens are essentially the same as those used at times by the Division of Physiology, National Institute of Health (Trembley 1946).

Chickens showing a high gametocyte count are selected for mosquito feeding. The chimney with mosquitoes is placed on the platform and fastened down with rubber bands. The sheet of pasteboard closing the bottom is then removed. The chicken with clipped back is placed back down on the bobbinet chimney top and held in place by means of a cloth wrapping and rubber bands.¹

The mosquitoes are allowed to feed on the chicken for a half hour or more, or until those willing to feed have finished. Sometimes it seems that wrapping the chimney

¹ This technique originally described by Beckman in Science, 88, No. 2274, p. 114.

to exclude light causes quicker feeding. From 50 to 90 per cent of the females in the chimney may be expected to feed.

After the mosquitoes have fed, the engorged insects are removed to an empty chimney by means of a suction tube. Figure 3 shows the procedure described.



FIG. 2. ARRANGEMENT FOR OFFERING MOSQUITOES CHICKEN BLOOD
(Photo by Andre Kaas, Malaria Control in War Areas)

Several chimneys of mosquitoes may be fed on a single chicken. All mosquitoes fed on a particular chicken are placed together in a single chimney (not over 50 insects per chimney).

Single chimneys containing the insects fed on a single chicken are placed in petri dish covers provided with several thicknesses of blotting paper or other suitable filler. The chimney and dish are then placed in prepared metal holders and held there by rubber bands over the tops. The fed insects, securely held in the chimney, are properly labeled and held for future use.

As soon as the chimneys of fed insects are assembled, a cotton pledget wet with a



FIG. 3. METHOD OF REMOVING MOSQUITOES WHICH HAVE FED ON CHICKEN BLOOD

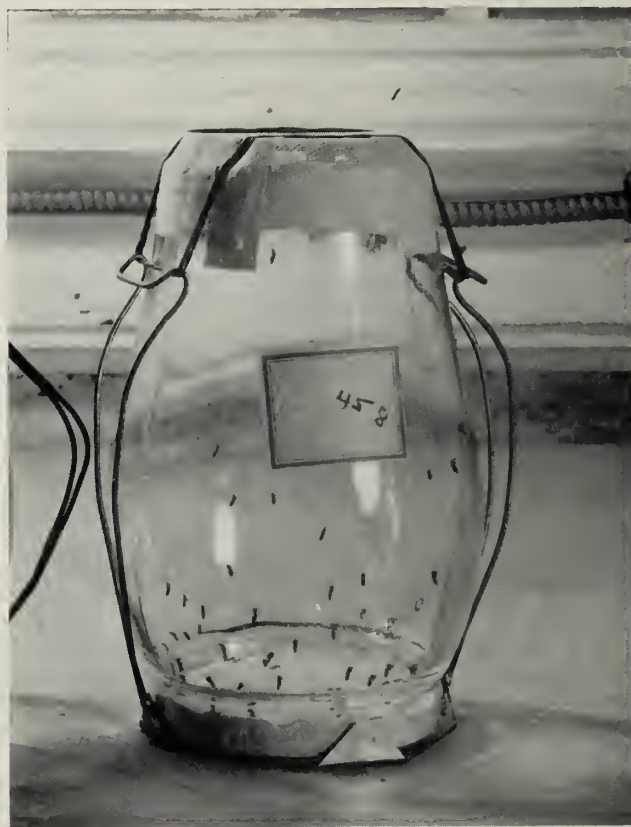


FIG. 4. DEVICE FOR HOLDING MOSQUITOES FOLLOWING BLOOD MEALS
Mosquitoes have remained alive in lantern chimneys for 110 days following feeding.
(Photo by Andre Kaas, Malaria Control in War Areas)

5 per cent sucrose solution (5 tablespoons sugar in 2000 cc. water) is placed on the bobbinet top. The solution is renewed every day, and new pledgets are used every other day.

While it is customary to feed mosquitoes on chicken blood on the fourth day following the collection of the pupae, it has been found that insects that do not feed readily at this time will often feed freely the following day if held over without any further moisture being applied.

Mosquitoes are not fed on chickens in this insectary on Saturdays and Sundays. Therefore, no female pupae are collected for this work on Tuesdays. Collection of pupae are made on Saturday and Sunday, as usual, as the adults feed on the Wednesday or Thursday following.

Disposition of Fed Insects

The blood-fed insects in the chimneys, fed sugar water as described, will normally develop sporozoites in the salivary glands in from 12 to 20 days. After the 10th day a few are examined for sporozoite density. When a proper density has developed, usually from 12th to 15th day, a desired number are used to prepare sporozoite inoculations. The remainder of the fed insects are held in the insectary as a reserve supply.

Length of Life of Aedes aegypti

The lantern chimneys containing blood-fed, but unused insects are kept in the insectary until the mosquitoes die. It has been observed that some insects fed one blood meal 4 or 5 days following pupation, and supplied thereafter with sugar water solution, have remained alive in the chimneys 110 days following the blood meal.

A study of the longevity of blood-fed female *Aedes* mosquitoes, kept in lantern chimneys as described, indicates that the mortality rate is very low—not over 15 per cent up to about the 21st day. There is then a rapid and pronounced increase in mortality, and only a small per cent of the original group (about 25 to 30 per cent) remains after a month.

SUMMARY

The techniques herein described for the rearing and handling of large numbers of *Aedes aegypti* have been developed over a period of 18 months. Emphasis has been given to the development of simple procedures involving the use of readily available apparatus and materials. The employment of simple practical methods for handling and feeding the developing insects has enabled the labor involved to be reduced to a minimum.

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RELATIVE EFFECTIVENESS OF VARIOUS REPELLENTS AGAINST *ANOPHELES FARAUTI* LAVERAN

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Tests against biting Diptera have shown a wide variation in the reaction of different species to repellent chemicals. In an effort to determine which materials were most effective against *Anopheles farauti* Laveran, a selected series was tested in the South Pacific. These materials had been shown to be good repellents either against various *Aedes* species or against *Anopheles quadrimaculatus* Say (Travis, Morton and Cochran).

Methods: Nearly all the data in this report are from tests made on Guadalcanal; some however, are from work done on Efate. At Guadalcanal, all experiments were made in small screened cages 24 x 24 x 24 inches. Three to five minute exposures were made at intervals of approximately 10, 30 and 60 minutes until the experiment was terminated. At Efate the tests were made in a large screened insectary (approximately 10 x 10 x 10 feet). Men with treated limbs exposed themselves in the insectary for 10 minutes immediately after treatment and thereafter hourly until the tests were terminated.

This large exposure room made possible excellent paired tests by treating one appendage with a standard chemical, R-612, and the other with a chemical for comparison. With few exceptions, tests were terminated when bites were either numerous or were received during two or more exposures. A "+" after the time elapsing until the first bite indicates that, in some instances, no bite had been received when it was necessary to terminate the tests.

The biting rates at Efate, which ranged from 2 to 20, were made by counting the number of mosquitoes on an untreated hand after a one-minute exposure. At Guadalcanal, counts of from 2 to 24 were obtained on an untreated arm after a one-half minute exposure. One milliliter of liquid repellents or one-half teaspoonful of creams was applied to the arm from the elbow to the wrist. When legs (knee to ankle) were treated, the amounts of materials being tested were increased by one-half. The temperature and humidity were sufficiently high that the skin was moist to wet in all tests.

Results: Of the 17 materials tested, table 1, undecylenic acid, n-butyl dl mandelate, ethyl dl mandelate and R-612 (2-ethyl-1,3-hexanediol) gave the longest repellent times, averaging 6 or more hours. The G.I. repellents varied considerably, R-612

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² Lt. C. S. Wilson assisted in collecting many of the larvae from which were reared the adult mosquitoes used in the tests on Guadalcanal, and two corpsmen, M. F. Abbitt, PhM3c, and L. F. Irving, PhM3c, assisted in making the tests. The tests at Efate were conducted with the colony at Malaria Control Headquarters. Major John C. Swartzwelder, SnC. AUS., Sgt. J. S. Haeger, AUS, and Pfc. T. G. Campbell, AUS, assisted with the experiments.

being much superior to dimethyl phthalate and Indalone. The 6-2-2 mixture (60 per cent dimethyl phthalate, 20 per cent R-612, and 20 per cent Indalone) was moderately good, with an average repellent time of a little more than three hours. Allyl cinnamate, alpha-n-amyl cinnamaldehyde and 4-tert.-butyl phenoxy ethanol burned the skin so severely that they were not tested.

The partial protection data, table 1, as shown by bites with repeated exposures at intervals of an hour, demonstrated that the better repellents gave greater protection after the first bite than did the poorer materials. The data on repeated exposures

TABLE 1

Summary of data obtained with various repellents when tested against Anopheles farauti Laveran. Guadalcanal and Efate, 1944

CHEMICAL	NO. TESTS	AVERAGE MIN. TO FIRST BITE	AVERAGE NUMBER OF BITES WITH EACH EXPOSURE (HOURS AFTER TREATMENT)										
			$\frac{1}{2}$	$\frac{1}{2}$	1	2	3	4	5	6	7	8	9
Undecylenic acid.....	4	432+	0	0	0	0	0	0	0	0.2	0.1	0.3	1.0
n-Butyl dl mandelate.....	4	411+	0	0	0	0	0	0.2	0.2	0	0.7	0	0
Ethyl dl mandelate.....	4	392+	0	0	0	0	0	0	0	0.2	4.0	3.2	
R-612 (2-ethyl-1,3-hexanediol).....	11	388+	0	0	0	0.2	0	0	0.1	0	0.1	0.2	
Repellent cream*	3	367	0	0	0	0	0	0	0.3	1.0	1.0		
p-Methoxy benzyl alcohol.....	3	252	0	0	0	0	0	0	2.7	5.5	1.0	5.0	
Methyl anthranilate.....	3	227	0	0	0	0	0	3.3	5.0				
iso-Propyl cinnamate.....	6	219	0	0	0	0	1.3	1.5	1.7	1.5	1.0	1.0	
2-Phenyl cyclohexanol.....	5	211	0	0	0	0	0.4	1.0	0.8	3.8	0	2.5	
6-2-2 Mixture†.....	8	198	0	0.1	0.2	0.1	0.2	1.2	0	0.3	1.0	1.0	1.2
8-2 Mixture‡.....	6	167	0	0	0.7	0	0	2.1	0.8	1.2	0.3	2.0	
Cyclohexyl benzoate.....	4	88	0	0.2	0.5	0.2	0	0.5	0	1.7	0.5	0	0
Indalone.....	4	72	0	1.2	1.0	1.7	1.0	0	1.0				
Dimethyl phthalate.....	10	43	1.6	1.0	2.3	1.0	2.0	1.0					

* Repellent cream composition:

Dimethyl phthalate 80% } 45%
R-612 20% }

Zinc oxide 52%
Calcium stearate 3%.

† 6-2-2 Mixture: 60% dimethyl phthalate, 20% R-612, 20% Indalone.

‡ 8-2 Mixture: 80% dimethyl phthalate, 20% R-612.

after the first bite are especially useful in the final evaluation of the better repellents. For instance, ethyl dl mandelate has little partial protection after the first bite, whereas R-612 with about the same repellent time is indicated to be a much superior repellent because of the small number of bites received on exposure subsequent to the first bite.

In one test at Efate, there had been no bites on a leg treated with R-612 when the test was terminated at the seventh hour. At the ninth hour, and after the treated appendage had been subjected to much rubbing, there were 10 bites on the R-612 treated leg and 85 on the untreated leg, a protection of 88 per cent.

In table 2 are results from paired tests, one leg treated with R-612 and one with a chemical for comparison. Tests were terminated shortly after bites were received on the leg bearing the test chemical. R-612 is shown to be much superior to dimethyl phthalate and iso-propyl cinnamate. Observations on these and subsequent tests showed that R-612 was somewhat superior to a zinc oxide cream containing R-612 and dimethyl phthalate.

TABLE 2

Summary of repellent times with paired tests against Anopheles farauti Laveran; Efate, 1944

MATERIAL	NO. TESTS	TIME TO FIRST BITE (MIN.)
Dimethyl phthalate	4	32
R-612	4	64+
iso-Propyl cinnamate	4	181
R-612	4	398+
Repellent cream*	3	366
R-612	3	352+

* Repellent cream composition: Dimethyl phthalate 80% } 45%
R-612 20% }

Zinc oxide 52%
Calcium stearate 3%.

TABLE 3

Relative effectiveness of several standard repellents when tested against different species of mosquitoes

SPECIES AND TYPE OF TEST	REPELLENT TIME (MIN.) WITH VARIOUS CHEMICALS			
	Dimethyl phthalate	R-612	Indalone	6-2-2 mixture
<i>Anopheles quadrimaculatus</i> * (laboratory)...	206	78	30	257
<i>Anopheles farauti</i> (laboratory).....	43	388	72	198
<i>Aedes aegypti</i> * (laboratory).....	234	363	160	321
<i>Aedes taeniorhynchus</i> * (field).....	153	276	164	212

* Data from Travis and Jones, August 29, 1944.

DISCUSSION

The tests with the G.I. repellents, R-612, dimethyl phthalate, Indalone and the 6-2-2 mixture confirm results and opinions of various workers and troops in the South Pacific. Other tests have shown that R-612 and Indalone were of little value when used against *Anopheles quadrimaculatus*, whereas dimethyl phthalate was shown to be an excellent repellent against this species. This situation was reversed in the case of *A. farauti*. It appears that data from repellent tests with one species of mosquito cannot be used to predict the effectiveness of a chemical against an unknown species. Thus, it is necessary to make tests with the various species con-

cerned to determine which is the most effective chemical. Mixtures of repellents, known to be good against several species, show more promise as a general repellent than any single chemical. Data demonstrating the variation in repellent time against different species is summarized in table 3.

In repellent studies, such as these, the data are presented in a manner to show the length of time until the first or subsequent bites. Various workers have designated the length of time to the first bite as "protection time" or "repellent time." Such data should not be construed to indicate the length of time that a repellent will give protection from mosquito bites under field conditions. The real value of these tests is to evaluate different materials by testing them under uniform conditions. Ordinarily, the field protection time is much less than that obtained in the laboratory.

SUMMARY

Tests with 14 chemicals and three mixtures show that under laboratory conditions, undecylenic acid, n-butyl dl mandelate, ethyl dl mandelate and R-612 give average repellent times of more than six hours against *Anopheles farauti* Laveran. Indalone and dimethyl phthalate were poor repellents against this species, with respective average repellent times of 74 and 43 minutes.

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MALARIA MORTALITY AND MORBIDITY IN THE UNITED STATES FOR THE YEAR 1945*

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Beginning with 1930 (Faust, 1932) the senior author, on behalf of the National Malaria Society, has made a yearly inquiry into the status of malaria in the United States. At first the data presented in the annual survey consisted of malaria deaths and death rates for the fourteen most malarious states in the southeastern part of the country. Later it seemed desirable to include certain additional political subdivisions contiguous to the highly malarious territory (Faust, 1937), and subsequently for the entire United States (Faust and DeBailey, 1942). In more recent years an attempt has been made to evaluate morbidity as well as mortality statistics.

During the depression years of the mid-1930's, a notable increase occurred in the malaria death rate, following which there has been a substantial yearly decrease in mortality in practically every malarious state. In the meantime more experienced laboratory diagnosticians have been trained for state departments of health, physicians have been educated in the desirability of accurate diagnosis, and as a result better malaria case reporting has been developed in many states.

During the recent War Years (1942-1945) large military centers were established in highly malarious as well as essentially non-malarious areas. While on maneuvers in such states as Louisiana troops were at first subjected to considerable exposure, although effective antimalarial measures were soon instituted within and immediately around the camps, so that this hazard was brought under control. With the return of hundreds of thousands of military personnel and thousands of prisoners of war from hyperendemic malarious areas overseas malaria of extrinsic origin was introduced into the country, particularly into concentration and separation centers. As of the end of 1945 many of the military cases of relapsing malaria have returned to their home communities, some on furlough, others on terminal leave and many more after discharge from the service. Thus, there has been superimposed on a decreasing native malaria problem a serious and widely scattered one of foreign origin. In the present report an attempt has been made to analyze this relatively complex situation, with the aim of providing not only a perspective of the 1945 status of malaria but also possible trends within the next few years.

As in previous years the basic data have been secured from the bureaus of vital statistics of each of the forty eight states, the District of Columbia and New York City. Grateful acknowledgment is made to all persons who have supplied information and particularly those individuals who have provided a separate breakdown of civilian and military cases by counties.

* Report of the Committee on Statistics, National Malaria Society, Miami, Florida, November 6, 1946.

PRESENTATION OF DATA

At the time of compiling this report detailed information on civilian mortality and morbidity has been received from all states and other political subdivisions except Arizona, Iowa and Pennsylvania, which have supplied no information.

In so far as the data have been made available the total malaria deaths certified by states for 1943, 1944 and 1945 and the total malaria cases reported for 1944 and 1945 have been incorporated into a table (Table 1). Similarly, in so far as county breakdowns of deaths and of cases have been provided in the returns these records have been allocated to their respective counties on outline maps of the United States (Figs. 1, 2). In this connection it must be pointed out that the maps represent deaths as such and cases as such rather than rates. The solid circles refer to military, prisoner-of-war and veteran data and the hollow circles to civilian records.

The 1945 civilian mortality figures continue to be highly satisfactory. They show a marked decrease in Arkansas as compared with 1943 and 1944; considerable reduction in Alabama, Florida, Georgia, Kentucky, Mississippi and South Carolina; essentially a status quo in Missouri, North Carolina, Oklahoma, Tennessee and Texas, and an apparent slight increase in Louisiana. The military and single prisoner-of-war deaths are geographically scattered and lack significance. The total number of reported civilian deaths for 1945 is 399 as compared with 584 in 1944 and 622 in 1943. The overall reduction of 46 per cent for 1945 vs. 1944 and 56 per cent for 1945 vs. 1943 indicates a continued favorable downward trend. Compared with 1935 (4268 certified deaths) the number of deaths has decreased 970 per cent. Meanwhile the population of the United States has increased perhaps 10 per cent during this decade.

The county distribution of malaria deaths is shown in Fig. 1. It indicates that hyperendemicity still exists throughout the southeastern coastal drainage from southernmost Virginia to central Florida; in the Mississippi drainage from Cairo, Illinois to Natchez, Mississippi; in eastern Oklahoma and Texas, and in the lower Rio Grande valley. Elsewhere the deaths are scattered and relatively incidental. It is quite possible that the four civilian deaths reported from Michigan and the one from Minnesota resulted from exposure in more malarious territory, either in the United States or overseas. On the other hand, the four deaths certified for northeastern Ohio and the two recorded ones from northeastern Kansas were possibly due to locally acquired malaria.

Only 16 counties had a death rate of 10.0 or more and only 3 counties had rates in excess of 25.0. These were as follows: *Arkansas*,—Phillips Co., 10.0, Union Co. 10.0; *Georgia*,—Miller Co., 30.0; *Kentucky*—Ohio Co., 12.3; *Louisiana*,—East Feliciana Parish, 28.0, Franklin Parish, 14.8, Sabine Parish, 20.3; *Mississippi*,—Carroll Co., 19.3; *S. Carolina*,—Darlington Co., 11.6, Orangeburg Co., 11.1, Williamsburg Co., 24.4; *Texas*,—Hidalgo Co., 10.4, Red River Co., 10.1, Shelby Co., 10.3 and Starr Co., 22.3. Miller Co., Georgia, East Feliciana and Sabine Parishes, Louisiana, Iron Co., Missouri, Williamsburg Co., S. Carolina and Starr Co., Texas either show increases in rates or at least indicate relatively important centers of moderate endemicity.

The malaria cases reported unquestionably provide an incomplete picture of the situation, but it has been deemed advisable to chart them by counties on a morbidity

TABLE 1
Malaria Mortality and Morbidity in the United States for the year 1945

STATE (OR OTHER MAJOR POLITICAL SUBDIVISION)	DEATHS CERTIFIED						CASES REPORTED						TOTAL CASES		
	Civilian			Military			Civilian			Military					POW
	1943	1944	1945	1944	1945	1944	1945	1944	1945	1944	1945				
Alabama.....	60	39	34				2271	2364	611	549		2882	2913		
Arizona.....															
Arkansas.....	108	117	43		3		138*	275	1427	1630		1427	1905		
California.....	3	8	0				2	3	1455	827	16	1583			
Colorado.....	1	1	0				1	1	32	296		62	297		
Connecticut.....	0	0	0				0	0	15	47		15	47		
Delaware.....	0	0	0				0	0	507	645		522	671		
Florida.....	41	33	22				15	26	102	20		416	460		
Georgia.....	37	34	26				314	440	1	39		2	39		
Idaho.....	1	0	0				1	0	251			274	51		
Illinois.....	9	3	6	1	2		23	6	237			80			
Indiana.....	6	10	3				80	51	78	860		241			
Iowa.....	1	2					4					91	870		
Kansas.....	3	1	2				13	10				27	54		
Kentucky.....	13	15	6		1		27	54				1469	1219		
Louisiana.....	29	29	34				ca734	567	ca735	652		0	28		
Maine.....	1	1	1*				0		0	28		23	614		
Maryland.....	0	0	1*				0	1*	23	613		572	1031		
Massachusetts.....	0	3	0	4	2		7	4	565	1027		242	477		
Michigan.....	3	0	4		4		24*	49*	218	428		60	380		
Minnesota.....	1	1	1	1			1	334	59	46		23,133			
Mississippi.....	74	61	39				22,398	18,860	735			223	426		
Missouri.....	25	14	14				41	65	182	361		28	30		
Montana.....	0	0	0				0	0	28	30		6	9		
Nebraska.....	0	0	1		1		0	8	6	1		4	5		
Nevada.....	1	0	0				4	5				2	5		
New Hampshire.....	0	0	0				0	0	2	5					

New Jersey.....	2	0	2	1 POW	1		14	99	812	1313		826	1412
New Mexico.....	1	3	0	1	2		6*		8 POW			14	
New York City.....	3	3	2				35	26	288	724		323	750
New York State.....	3	0	0				12*	8	218 POW	557		230	565
North Carolina.....	21	29	23				130	398	24	156		154	554
North Dakota.....	1	0	0				1*	6	7			8	6
Ohio.....	5	5	5				21*	3	133	107		154	110
Oklahoma.....	30	18	19				1359	1101	49	12		1408	1113
Oregon.....	0	1	0				22	2	428 POW	54		450	56
Pennsylvania.....	1	1		1			2		343			345	
Rhode Island.....	1	0	0				0	0	264	182		264	182
South Carolina.....	62	63	40				9478	8674	421	1185		9899	9859
South Dakota.....	0	0	0		1		0	0	0	7		0	7
Tennessee.....	24	15	20				185	117	7	79		192	196
Texas.....	54	74	49				7498		575			8073	7194
Utah.....	0	0	0				1	2	156	110		157	112
Vermont.....	0	0	0				0	0	0	3		0	3
Virginia.....	1	1	1				37	764	676	57		713	821
Washington.....	0	1	0				4	1*	4	11		8	12
West Virginia.....	0	1	0		1		3	0		166		3	166
Wisconsin.....	1	0	0	1			0	0	133	90		133	90
Wyoming.....	0	0	0				10	1	50	16		60	
District of Columbia.....	0	0	1				7	3	164	86		171	89

* Includes deaths or cases which acquired disease outside state.
 POW—Includes a few prisoners of war.

map (Fig. 2). The distribution and density of genuine autochthonous cases is probably indicated in the data obtained from only three states, South Carolina, Mississippi and Texas; elsewhere in the south the cases as reported are too few and too inadequately distributed to show the actual extent of present-day malariousness. It is both unnatural and improbable that malaria stops at a state line. For example, malaria is reported for every county in Mississippi, but many adjacent counties in neighboring states are reported as malaria-free or as much less malarious than the border counties of Mississippi. Topographically these adjacent counties of neighboring states are much the same, while the malaria control carried out in Mississippi

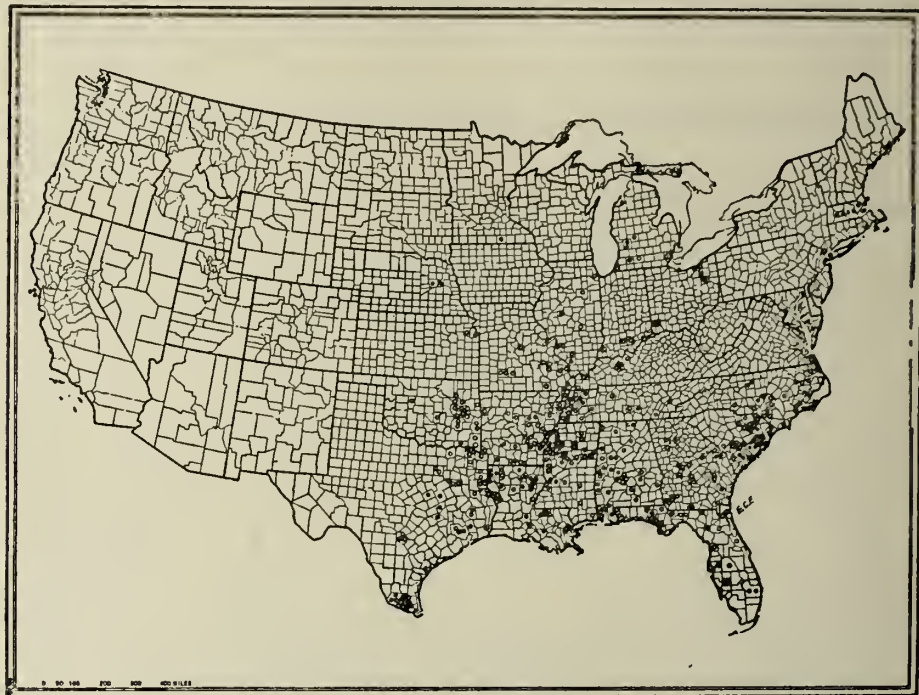


FIG. 1. Map of the United States showing malaria deaths in 1945 reported by state bureaus of vital statistics. Each circle represents one death. Hollow circles are civilian, solid circles are military personnel.

is at least the equal of that in nearby states. Probably the morbidity records for the three states referred to above are more accurate and more representative of the actual malaria situation than those in other southern states.

The distribution of malaria cases in the northeastern and north-central states and in California is particularly interesting. Sufferers from relapsing malaria who have been released from military service have become widely distributed throughout New England, New York, Ohio and Minnesota. Most of these states have allocated these cases to military personnel (solid circles on Fig. 2). On the other hand Minnesota appears to have classified them as civilian. Although malaria has been mildly endemic in recent years in the upper Mississippi valley, it is not widely

distributed throughout Minnesota. It is evident that veteran cases in this state have been reported as civilian.

In a considerable number of the states the high concentration of military cases represents malaria contracted outside the United States. In some instances, however, it is reported that the disease was contracted in malarious areas of the United States where the individuals had been encamped. Thus, the incidence map in so far as military cases are concerned refers to place of occurrence rather than to that of exposure. In this way malaria has been carried by patients into areas which were never malarious or have been relatively free of the disease for many years.

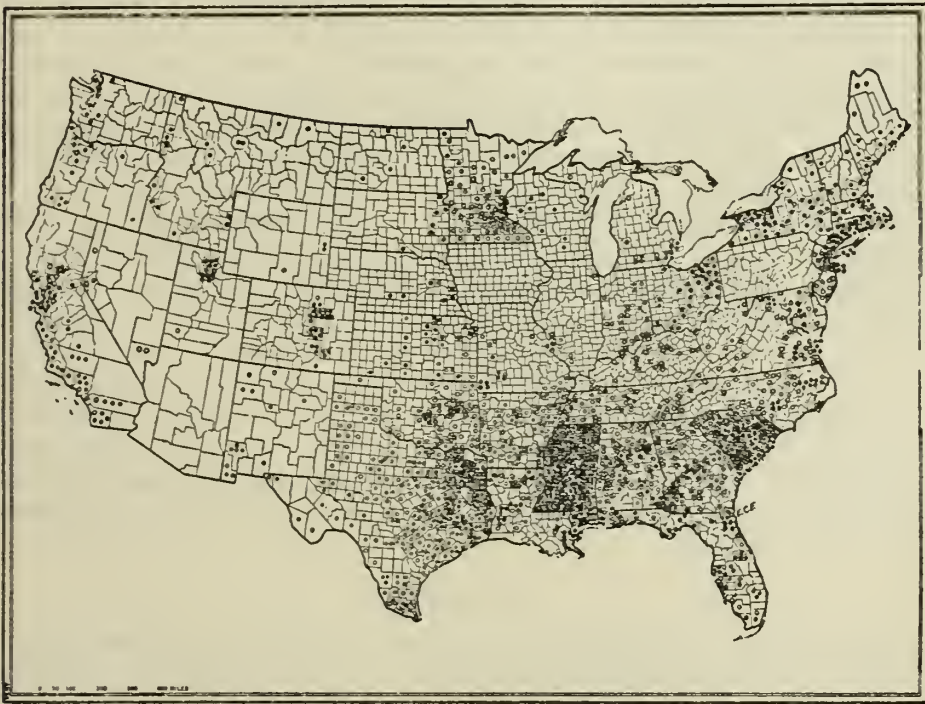


FIG. 2. Map of the United States showing occurrence of malaria in 1945 reported by state bureaus of vital statistics. One circle per county indicates one to 10 cases; two circles, 11 to 25 cases; three circles, 26-100 cases; four circles, 101 to 500 cases; five circles, more than 500 cases. Hollow circles are civilian, solid circles are military personnel.

Several of the state reports indicate that malaria has occurred in the civilian population as a result of the transfusion of apparently uninfected donor's blood. Statistically these are not important but they provide evidence of a continued hazard of transmitting malaria by blood transfusion if the donor has ever had a history of malaria. On the other hand, it appears that malaria propagated by drug addicts is on the decline. Although the reports are incomplete, the use of malaria as a therapeutic procedure for neurosyphilitics in state institutions appears to be on the increase. Several hundred cases are reported for 1945, while a considerably larger number were not notified to state boards of health.

DISCUSSION

If World War II had not occurred it is probable that malaria in the United States would have shown a satisfactory annual decline as a result of control measures instituted some years earlier. However, when large bodies of military personnel were concentrated in training areas in malarious or potentially malarious areas, intense antimalarial campaigns were instituted within and immediately around the camps. This tended to reduce exposure of the military groups and at the same time was reflected in an appreciable reduction of malaria among civilians in the surrounding areas, a reduction considerably greater than might have been anticipated had not special efforts been made. Although some trainees in heavily malarious areas in the United States acquired the disease and carried it to training centers in less malarious parts of the country, there is no evidence that malaria was established in the civilian population as a result of this type of exposure. More recently the return of many thousands of relapsing malaria cases from hyperendemic areas overseas and the dispersal of these individuals to almost every county in the United States have provided a potential source for the reestablishment of the disease in regions which have long been malaria-free and are not conscious of the danger. This possibility is the more likely since malaria in veterans is almost exclusively *vivax* in type and is therefore capable of developing in practically every part of the United States where anopheline mosquitoes breed. Moreover, it has been adequately demonstrated that the prevailing anophelines are good biological vectors of Mediterranean, Southwest Pacific and China-Burma-India strains of *vivax* malaria (Young et al., 1945). The development of malaria in the civilian population as a result of association with veterans who acquired *vivax* malaria overseas has already been authenticated. This may occur many times in different parts of the country without serious increase in the overall amount of malaria but it constitutes a hazard which should not be ignored.

SUMMARY

This constitutes the sixteenth consecutive annual report to the National Malaria Society on the status of malaria in the United States. Analysis was at first limited to deaths and death rates in the more highly malarious southern states, but its scope was gradually enlarged to include both mortality and morbidity for the entire United States. In so far as state department of health records have been made available the report for 1945 provides information by major and minor political subdivisions on the mortality and occurrence of malaria in both civilian and military populations. Practically all, but not all, of the reported military malaria was acquired overseas and much of this is now widely distributed throughout the country as relapsing *vivax* infection in veterans who have been discharged from service.

Malaria mortality in the civilian population continues to show a very satisfactory decline. Within a decade it has been reduced approximately nine-tenths. Yet there are areas of hyperendemicity remaining in the southeastern states, in the Mississippi valley, in eastern Oklahoma and Texas, and in the lower RioGrande. Most of malaria case reporting is still inadequate to provide satisfactory evidence

of the accurate distribution and intensity of the disease. South Carolina, Mississippi and Texas constitute exceptions to this general observation. Certain states, notably those in New England, New York, Ohio and Minnesota, all of which are regarded as essentially non-malarious, now have widely disseminated cases of malaria in military or veteran personnel. Malaria in veterans in California is also considerable. This may provide a means of reestablishing malaria in many areas which have been essentially malaria-free for many years.

Acknowledgment. Sincere thanks are extended to Mrs. Anne Richards, Department of Tropical Medicine, Tulane University, for loyal and intelligent assistance in securing and assembling the data which have formed the background of this report.

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AUTOMATIC SIPHONS FOR ANTIMALARIAL CONTROL OF TROPICAL STREAMS

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Among methods for the control of mosquito breeding in rural streams in the dry season, the use of artificial flushes to promote turbulence and variation of water surface elevation promises to be an economical one for a small continuous stream.

In coastal areas of the West Indies it has been observed that with the advance of the wet season there is a gradual movement of *Anopheles aquasalis* breeding from permanent marsh to temporary swamps. In the dry season a similar movement takes place upstream from the coast on the sides of streams that have a steady flow. The scarcity of breeding in these same streams, when they are thoroughly flushed in the wet season and the certainty of breeding within that same season in the more tranquil permanent saline coastal marshes, suggest that the *aquasalis* mosquito prefers saline water for breeding and that the saline marshes might be the natural permanent home of the species with secondary and less breeding taking place in fresh water.

The late Mr. Shannon of The Rockefeller Foundation (International Health Division) pointed out that although *aquasalis* breeding is heaviest in the brackish waters along the coastal areas, its absence in streams during the wet season is due to the mechanical fluctuation and turbulence of the streams rather than to their lack of salinity. The automatic siphon offers a method of producing the necessary turbulence in the dry season.

The siphon is an old device applied to lift water over any obstruction and to carry it to a lower level through a raised closed passage, from which all air has been exhausted. This device is now used in antimalarial work to empty the water collected from a continuous small flow which has been stored behind a dam, to give intermittent heavy discharges, which flush the stream below the dam and inhibit mosquito breeding. This action must be automatic for economical mosquito control.

The flushing of a stream by the automatic discharge of a large body of water, at intervals, through the medium of a siphon, has two main effects:

(A) The delicate eggs of the mosquito are damaged as they are thrown up and stranded on the banks, the rocks, or the stems of grass or reeds. The interval of a few hours between the flushes is long enough to ensure complete drying of the eggs and their consequent destruction. The frequently repeated flush exerts its most damaging effect on the eggs, for when it is used few larvae appear. Newbold and Cochrane (1943) found that frequent and careful dipping for larvae in the stream

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immediately below the Grenada siphon gave negative results despite the fact that above the siphon, breeding was present and deliberately not controlled. The more frequent the flush, the more hazards the eggs and larvae have to overcome.

(B) The stream becomes turbid. Earle (1936) is of the opinion that in sluiced channels, particularly in fairly flat country, the soil disturbance and the liberation of silt has a deterrent effect upon larval breeding. Opinions are divided on the question of turbidity alone as a mosquito control. The character of the silt and the particular species of mosquito may have a bearing on the question. Shannon's experiments in Trinidad to determine the effect of turbidity on *A. aquasalis* breeding did not furnish convincing proof that turbidity alone can be used as a control for that species. Covell reports that silt in suspension is in certain cases fatal to malaria-carrying anophelines.

The total volume of water and the rate of its discharge naturally has an important bearing on the efficiency of the flush as an antimalarial measure. Ramsay (1940) states that a prolonged flush controls a greater length of channel than frequent but shorter flushes. In northern Bengal he considered that a total discharge of 50,000 imperial gallons¹ was the minimum amount necessary to control a mile channel 6 to 8 feet wide. In Grenada we ascertained that a flush of approximately 5,000 imperial gallons in 10 minutes controlled a 2- to 6-foot-wide stream over a distance of 400 yards. The Grenada siphon (shallow seal) had a cross-sectional area of $1\frac{1}{2}$ square feet. In Tobago a smaller flush, from a deep seal siphon, of 3,500 imperial gallons from a concrete cistern collecting water from a spring, discharged in $4\frac{1}{2}$ minutes, averaging 2.15 cusecs.² The Tobago flush occurred every nine hours, and while the results were good they were not perfect. A few larvae, mostly first and second stage, and one pupa were found under overhanging ledges of pools at bends after a most thorough search; none were found close to the siphon. Measures were taken to streamline the ravine at these overhanging ledges in the pools, with good results. Later searches revealed no breeding. We consider that if the flush had been twice as large and more prolonged, even this work would not have been necessary. The ravine was rocky and winding, with frequent gravelly pools, and the flush was effective for over 2,500 feet. Had the shallow seal type of siphon been used here, the actual flush, with the water left available after the priming water had exhausted the air in the siphon, would have been too small to be effective.

At Morvant in Trinidad, B. W. I., a 24-inch diameter siphon (deep seal) was built with the simple but carefully designed automatic control devices to be described later. The water was collected from about one thousand acres. The average discharge was not far short of 20 cusecs and lasted for 10 minutes. Practically 70,000 imperial gallons of water were discharged per flush, and the 6-foot-wide stream was raised 18 inches below siphon discharge, 17 inches 2,000 feet down stream, 15 inches 4,000 feet down stream, 13 inches 6,000 feet down stream, 8 inches 8,100 feet down stream, and 4 inches 10,650 feet down stream. The wave took 1 hour and 20 minutes to reach this last station. This siphon worked automatically without the slightest

¹ Imperial gallon = volume of 10 pounds of water at 62°F.; equivalent to 1.2 American gallons.

² cusec = cubic foot per second.

attention through the critical first dry 6 months of 1945, where previously heavy breeding had occurred annually. Routine searches biweekly and special investigation revealed no breeding.

Experiments were made by Shannon to test the efficiency of the siphon. One experiment involved the breeding of 2,000 *Anopheles aquasalis* larvae in The Rockefeller Foundation laboratory and their release in various parts of the reservoir, including the flooded stream margins. After the siphon had discharged, diligent search by six men, operating independently for hours, located only 1 larva above the siphon, none down to the 8,100 foot station, and 3 first-stage larvae between the 8,100 and 10,600 foot stations. The siphon is a complete success in controlling dry-season stream breeding, and the capital cost is only a fraction of what paving the stream would have been. The maintenance is practically nil.

Where the water in a stream fails and the watercourse becomes only a series of unconnected pools and finally dries up altogether, it is obvious that flushing is impractical. The perennial stream is ideal for a siphon; but even if there is a month's break in the continuity of the stream, the antimalarial control by a siphon flush might still be effective, since renewed breeding, if any is introduced from uncontrolled breeding areas within effective flight distance, will soon be eliminated with the return of the flushing water; and the density of the adult mosquitoes may not, in the meantime, have become dangerous. The constant feed of water and the type of waterway for which a siphon is being considered, must be studied before a design is prepared.

The siphon installed at Tempé, Grenada, B. W. I. (Cochrane and Newbold 1943), following the practice developed in the East, had a wide rectangular passage and a shallow seal. Considerable time (approximately equal to the length of time of the heavy flush) was taken to prime the siphon, with consequent escape and reduction in the volume of flushing water. Blacklock (1939) showed this also by experiments. The low rectangular throat is necessary with a shallow seal in order to get a wide surface for the entrainment of the air.

Blacklock also stresses that often because of the isolated location of the flushing apparatus, the action should be fully automatic. Covell (1941) points out that siphons developed in Asia need frequent attention; possibly debris interferes with the vacuum break. The Morvant siphon, described later, has controls which eliminate this source of trouble.

A deep seal design was put in at Darrel Spring, Tobago, B. W. I., for use with an abandoned water supply cistern which previously served to collect water from the spring for domestic purposes. The cistern was 10 by 16 feet, with a depth of $4\frac{1}{2}$ feet. The overflow dribbled down a rock ravine with frequent bends and pools for about half a mile to the sea. The ravine was a bad anopheline breeding place in the dry season. The development was based on the dosing siphon used in sewerage work, rather than the surface regulation siphon employed in water reservoirs. A seal depth of 30 inches was used, with a concrete bell over the inlet. The siphon diameter was $7\frac{1}{2}$ inches, as a balance had to be maintained between the capacity of the flush and the available flushing water. The flush occurred about every 9 hours

and was maintained for about $4\frac{1}{2}$ minutes. The measured discharge rate averaged 2.15 cusecs. The deep seal design allowed a circular discharge pipe to be used.

The vacuum-breaking device employed at Darrel Spring was that normally used in sewerage work—a pipe with a turned-up elbow near the bottom of the bell, leading to the top of the throat. No trouble was experienced in priming or vacuum breaking; the water was debris clear, in contrast with the stream water at Tempé, Grenada, which had many leaves and floating matter to interfere with the original vacuum-breaking device. While priming always occurred, it happened at variable elevations within a few inches, depending on the manner of the air escape at the critical elevation of the rising of the water within the bell near the crest. There was no pilot seal.

When the water to be siphoned is clear of debris, the vacuum-breaking device of a turned-up elbow and guard of suitable area and design will work well. But if debris



FIG. 1. STREAM BELOW SIPHON WITH 24 INCH STAKE--DRY SEASON

and floatage are present in the water, the action is uncertain even when there is a screen. In the large siphons used in open streams, the writers have developed an auxiliary control siphon from within the main siphon, with a reduced outlet to the free air down stream. It needs neither strainer nor screen and is free of the main flow through the siphon; it offers no obstruction to the flow.

This newer design, as used at Morvant, allows the concrete of the siphon throat to be under air pressure the minimum of time. (Figs. 1-4)

A diagram is given (Chart 1), based on working drawings of the 24-inch siphon at Morvant (1944). It illustrates the automatic control and operation of the siphon at different phases. In Figure 4 the control siphon has just started to operate. As this, in practice, may need a small adjustment to assure the absolute minimum loss of water, an adjustment is provided. The outlet of the control siphon is reduced to the smallest size and is a few inches higher than the main siphon inlet lip. Im-



FIG. 2. MORVANT SIPHON IN OPERATION, SHOWING ONLY 6 INCHES OF STAKE
(THE LATE MR. SHANNON WATCHING THE FLUSH)



FIG. 3. SHOWING UPSTREAM ENTRANCE TO SIPHON ON LEFT BUT WITH COLLECTING PLANKS TAKEN
AWAY FOR WET SEASON PASSAGE OF GRAVEL AND DEBRIS.
VIEW LOOKING DOWN STREAM

mediately air is quietly allowed to enter the main siphon undisturbed by the action of the water in the reservoir, finally completely breaking the vacuum in the main siphon.

One of the practical aspects of siphon operation in the West Indies for mosquito control is the tendency of the rivers and streams of the islands to bank gravel, sand,

and silt against any obstruction placed across a river. There is a comparatively short flat run to the sea after a steep fall from the hills. Water reservoirs have been completely filled with gravel in spite of frequent flushing by a gate. This has been provided for in our work by a major passage for the streams when in flood, at which time the control of mosquito breeding is not necessary. Stream breeding is only found in the drier months of the year when the runoff is steady and small, giving very stable stillwater margins.

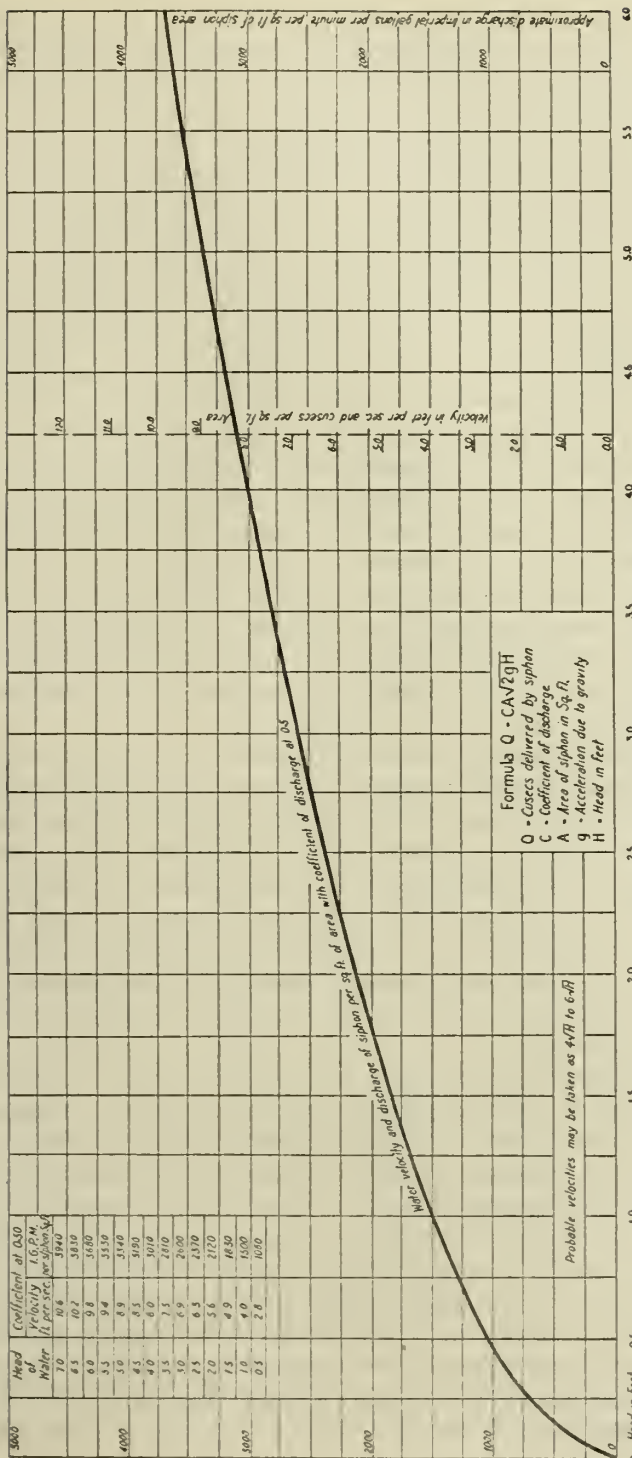
Photographs show how the siphon is placed well to the side of the stream, allowing full stream passage in the rainy season. Roughly speaking this opening is closed for the first six months of the year and opened for the second six months.



FIG. 4. MORVANT 24-INCH SIPHON IN OPERATION IN THE DRY SEASON, FLUSH AFTER SIPHON.
VIEW LOOKING UP STREAM

A chart has been prepared showing the variation in velocity with the altering head of water through the siphon. A coefficient discharge of 0.5 was taken for the curve, but siphons designed on the lines advocated will always have a higher coefficient. The curve shows a great reduction in velocity when the head falls to about 1 foot. It is useful in estimating the discharge per square foot of siphon area. Discharge coefficients up to 0.75 may be expected.

Considering the detail factors of the design for each part of the siphon, the nominal size of the siphon must have a relation to the dry weather flow of the stream. It should always have a discharge capacity lower than the average storm discharge to avoid damage to the banks. The velocity through a siphon for the purpose of fixing this size may be taken as 4 feet a second. The average velocity of a mountain stream may be taken as 1 foot a second. Therefore to get a flush of four times the dry season flow, the siphon area should at least equal that of the area of the dry season stream. The siphon area could with advantage be much larger and the minimum discharge of a siphon for a small stream should be 5 cusecs.



CURVE OF POSSIBLE SIPHON DISCHARGES

CHART 2

The duration of the flush, to maintain the height of the wave, should be long enough to counteract the flattening-out effect caused by the resistance of the stream bed. Ten minutes should be the minimum time, and a longer time would be useful. A longer flush has a tendency to carry further. A reservoir which can collect a day's flow will carry the flush further, through the same siphon, than a reservoir which can only collect one hour's flow. The reservoir must have a capacity to keep the flush going the minimum effective period. If the stream bed in itself above the siphon does not have this capacity, it can be augmented by a low dam on the nearby banks, or the bed above the siphon can be excavated. The bed of the stream at all parts within the reservoir should be opened down to the level of the siphon inlet and given a flow capacity at the siphon end somewhat more than the terminal flow through the siphon at the low head. A waterway cross-section is suggested of ten times the area of the siphon.

In the East, where labor is cheap, earthen dams, expected to be renewed each year, have been advocated. Together with these temporary dams a standardized precast siphon is used. In the Western world, where wages and living standards are higher, it is necessary to put up more permanent works for mosquito control. Concrete or masonry dams should be used. The dam must be low to be economical and must have a paving on the downstream side, to take the overflow of floods which must overtop the dam at times. In connection with masonry dams an adequate cutoff wall below the dam is advisable to hold up any water running in the gravel bed below the surface. In the dry season the addition of this underflow helps the antimalarial flush.

Unnecessary height of the dam should be avoided for reasons of expense and safety. In the dry season the river is completely blocked to collect all possible water. Even then, provision must be made for a flash flood. The top level of the collected water should be within an inch or so of the top of the planks used for dry season collection; and a sudden storm would be able to top it and even go over the siphon head, which would be a few inches higher. But definite provision must be made with the area and gradient of the river bed for the wet-season operation in order to prevent flooding above the siphon and the accumulation within the reservoir of gravel and sand brought down by the storms. The waterway must be in proportion to the highest runoff of the area.

The paving of the river through the dam and below the siphon should be on the gradient of the river and dish slightly to the center of the bed. No paving is necessary before the inlet to the short leg, but paving should be carried down past the outlet of the siphon for eight siphon diameters to allow the flush to steady somewhat and avoid excessive erosion of the bed. The waterway immediately below the siphon must be of ample width to allow all the flush to get away easily without unduly raising the level of the water at the discharge. If the water level does rise, it causes a loss in the available head through the siphon and a lowered discharge. In all streams, the position of the siphon is better at the side of the stream in order to give a straight run through for the wet season flows and to allow clear passage for heavy debris and stones. In mountain streams, gravel and silt will accumulate where an enlargement of the bed occurs, as at a reservoir. The wet season opening

aids the clearing of this shoaling and reduces the annual clearing. The siphon is only put into operation at the time of regular low flows.

The drawdown is the difference between the initial and final elevations of the water in the reservoir during the operation of the siphon and is largely determined by the surrounding country and the capacity of the needed reservoir. Associated with this question is the site of the siphon. The best site is at the end of a long flattish reach of the watercourse, partly because it gives a good reservoir, but also because the heavier silt will tend to drop before it reaches the siphon. If the watercourse has cut a deep bed for itself, a shallow drawdown is unnecessary. A deep drawdown means a higher, thicker and more expensive dam. In flat country, wing dams may have to be run sideways from siphon to higher ground to gain reservoir capacity. In such country a rise of water level of even 2 feet in the bed of the stream might interfere with up-stream drainage. In this connection it must be remembered that a flushing siphon will go into operation only in fairly dry weather, when extreme drainage above is not so necessary. Flushing siphons can be made to operate with a drawdown from 12 inches to 60 inches or more, depending on the economical depth. With the advocated design of the control seal and control siphon, drawdown can be made positive.

By reference to the diagram of velocities it will be seen that a velocity of 9 feet per second is not too high to expect in a fair-sized siphon with an initial discharging head of five feet. Some friction loss occurs at this high velocity as well as a loss of entry. It is necessary, for efficiency, to make the part with the highest velocity circular in order to obtain the best hydraulic section. That is the great advantage of the deep seal and the control seal. A deep throat can be made with a smooth transition from the large area short leg with low velocity to the circular higher velocity long leg. The short-leg area must be very much larger, as will be shown later; but the entrance to the short leg has to be low and confined. This is partly to get as much drawdown as possible. The opening under the short-leg inlet should not be more than 14 inches in height in order to allow a man to get inside the short leg for smoothing, air sealing, and examination. The velocity through this inlet should not be more than 3 feet per second, so that taking 9 feet per second as a fair maximum velocity the area there must be 3 times that of the area of the long leg. Nothing much is gained by making the inlet lower, as the terminal head of the working siphon drops to about a foot and at that head the velocity begins to fall sharply.

Siphon spillways constructed for large waterworks reservoir surface control are usually made with a priming step or a shallow seal. A considerable passage of water is necessary to exhaust the air from the siphon and establish siphon action. The object of these surface-regulating siphons is to get rid of any and all surplus water above a safe level. Antimalarial work dealing with siphons for small flows cannot afford this waste. The object is to put all available water to work by giving a strong flush to the stream, not just getting rid of surplus water. The deep seal type of siphon made of iron as used for sewer flushing avoids this preliminary water passage. The early types of siphon for antimalarial work had shallow seals, of the waterworks surface control type, and quite a fair amount of the collected water was wasted in carrying away the trapped air from the siphon to establish the necessary vacuum.

The deep-seal type for antimalarial work involves a concrete structure to be reasonably airtight at pressures up to 1 pound per square inch. This is possible, as a $7\frac{1}{2}$ -inch siphon with a concrete bell crown having a seal depth of $2\frac{1}{2}$ feet is in successful operation in Tobago. The concrete bell was made especially dense and was bitumen coated (colas) on the inside.

The deeper the control seal is made, the deeper the throat can be with efficient priming. The depth of the throat can be that of the controlling seal. With any siphon, that means a nearer approach of the throat form to the efficient circle. In large river siphons this is also important in that it allows passage to solids that get into the short leg. However, increasing the depth of the controlling seal prolongs the time the concrete siphon is under pressure. This condition has not proven a drawback so far, but it is conceivable that a concrete siphon could not hold air pressure indefinitely. Every means of perfect construction joints, dense concrete mixtures with graded fine aggregates, and the thorough sealing of the inner surface is advisable with deep seal siphons. If the drawdown is 3 feet and the seal 1 foot, it may happen that because of the increased volume and surface at the upper levels of the reservoir, the time to raise the last foot will be more than that required for the first 2 feet, with the possibility of air leakage.

For convenience the three stages of automatic siphonic action may be classed as:

(A) The period from the first collecting level (i.e. the bottom of the drawdown) until the inlet to the control siphon is covered.

(B) The period of compressing the air in the siphon to hold back the water in the siphon and gain a good head for priming.

(C) The period from the release of the control seal, during discharge, until the vacuum is broken by the inlet of air at atmospheric pressure.

In order to obtain a good throat to the siphon and yet not have an excessive B period, 12 inches has been adopted for 24-inch siphons and smaller. The object of the control seal is to hold back the water inside the siphon by entrapping the air until the head of water positively ensures priming when the air is allowed to escape. Therefore the air connection to the control seal should be at the last collecting place of the air in the siphon. In our designs we place it at the back of the throat and at an elevation half way up the throat. The actual pipe to the seal need not be more than 1 inch in diameter, as the up leg of the control seal must be small enough to be completely cleared of water with low air pressures. A certain time must elapse for the escape of air from a large siphon through a 1-inch pipe. This period of air escape-checks the surge of the rising water and thus the siphon crown will not be subject to shock.

To prevent the re-entry of water into the control seal as the air pressure is reduced when the B period is changing over to the C period, the control seal exit must be above the body of water in the main seal well. To ensure that the control seal always remains full of water at the end of the C period, the seal should have a 2-inch diameter enlargement at the lower end of the down leg of the control seal, but not at the up leg, which must remain 1 inch. For the convenience of a slight adjustment in the height of the up leg, to deepen the control seal if necessary, the top end of the up leg can have non-tapered screw threads, long enough to take a collar and lock

nut, and be flush with the top, the actual pipe of the up leg being $\frac{1}{2}$ inch short of the seal depth. The adjustment to raise the top water-discharge level of the reservoir a trifle is advisable to ensure that the control siphon is always filled and will operate full bore just before the control seal is cleared of water. The main seal depth for the siphon must always be a little deeper than that of the control seal.

The control siphon is arranged by the position of its inlet to delay the start of period B until the water in the reservoir is nearly up to the crest of the main siphon. The distance below the crest when the rising water will completely close the control siphon connection and start compression will be discussed later.

Any vacuum-breaking device placed on the upstream side of the siphon in an open stream is liable to derangement by debris; it also has to be of ample proportions with large siphons and be sufficiently high above the surge of the water to operate completely without the necessity of admitting air below the short-arm inlet. We have found that the most satisfactory method of ensuring a position break in the vacuum, is to have an auxiliary control siphon of small size, with the long-leg outlet downstream of the siphon and a few inches (say 6 inches) above the level of the main siphon inlet. This control siphon can be small—1 inch in diameter and reduced at the bottom of the long leg to $\frac{1}{2}$ inch in diameter. The crown of this siphon is high, the same level as the main siphon crown, and in operation is arranged to fill completely and only function as a siphon the very shortest time before the control seal releases the compressed air. The adjustment on the control seal makes this possible.

The loss of water from this control siphon is negligible. Because its long leg is shorter than the short leg of the main siphon, the control siphon will cease to operate before the main siphon has drawn down to the lowest reservoir level and will begin to admit air to the main siphon. This will slow down the main siphon and soon check it completely. Air may or may not enter below the short leg of the main siphon, but the process of admitting air will be continued through the control siphon until pressure within the main siphon is in equilibrium with that of the atmosphere no matter what happens on the upstream side. With these controls the opening and closing of the C period are carried out gently without shock.

The long leg of a siphon, having the smallest area, determines the nominal size of the siphon. It has been shown that in no other part except the throat should the area of passage be less than three times the nominal size. The throat should have a streamlined transitional form from the larger short arm to the long arm. In the highest part, where air might collect, it should be only slightly larger than the long leg in order to sweep and carry air along with the stream. The form should be that which will aid this action. Air entrained in slow moving water tends to separate from the water and collect in the highest part of the throat. This is not likely to happen until the velocity falls to about 2 or 3 feet per second, that is, toward the end of period C.

The relative area *on the horizontal* of the short leg of the main siphon to that of the area *on the horizontal* of the long leg, determines the major factor of the necessary vertical movement of the water in the short leg to compress the entrapped air, that is, the distance the control siphon inlet is below the crest of the main siphon. We advocate the ratio of six to one in the areas. This materially reduces the hydraulic

losses in the short arm and allows the small movement of 2 inches upwards of the water in the short leg with a 12-inch seal to displace the seal volume in the long leg.

A further allowance of upward movement must be arranged to compress the entrapped air. The air must be increased in pressure about 3 per cent to balance 12 inches of water and since in the 24-inch design which we take as a standard type, there is about ten times air volume to be compressed, to the main seal water volume displaced, we must add 30 per cent to the upward movement, that is, about $\frac{5}{8}$ of an inch. The level of the water in the short arm will then rise about $2\frac{5}{8}$ inches when the air connection through the control siphon is closed. Making the distance of the air connection 3 inches below the crest will allow a matter of a $\frac{3}{8}$ -inch possible adjust-

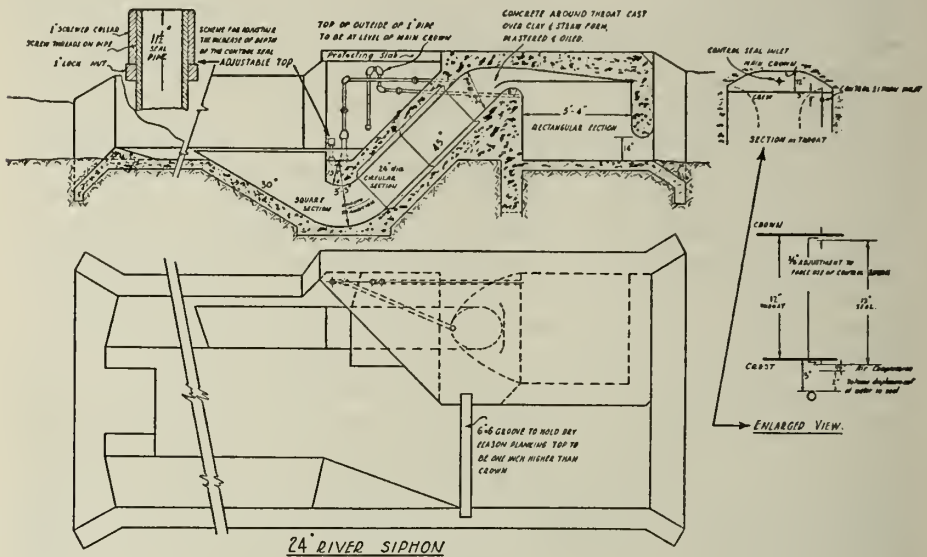


CHART 3

ment to the control seal, to force the discharging level to just top and fill the control siphon and the main siphon crown. The throat depth is 12 inches, based on a nominal control seal depth of 12 inches.

The long leg must be uniform in size and shape to disturb the stream of water as little as possible. No flare in the part of the seal displaced can be allowed, as that increases the volume to be displaced. The seal releasing well can have a gradual increase in area to reduce hydraulic losses. The highest velocity and friction will then be confined to the long leg. For this and constructional reasons, the shape should be circular in cross section and with a smooth surface. This design allows the entry of a man to smooth irregularities and use three coats of emulsified bitumen on the inside of the short leg and throat for air tightness. Close consideration of these fine points in design will raise the coefficient of discharge up to 60 and 75 per cent with corresponding beneficial results in service of a better and stronger flush.

Siphons can be placed in series in the same stream if breeding is along all reaches

of the stream. It is advisable to build one first and observe the effective control length suitable for the stream.

Siphons can be put in parallel, one on each side of the stream. This might be necessary to get an augmented flush within a certain reach. In this case the crests and throats must be exactly level with each other and there must be a connecting air pipe of fair size to equalize their air pressures. A set of controls on one siphon alone can be then used to serve the two siphons.

The final drawing shows a working design on these lines of a 24-inch siphon. Designs have also been prepared for 12-inch and 36-inch siphons. A 36-inch siphon is now under construction with an 18-inch seal for a river which is 25 feet wide.

The authors record with regret the death of Mr. Raymond Shannon, staff member of The Rockefeller Foundation in Trinidad. Mr. Shannon gave invaluable help, suggestions, and inspiration to the authors during their work on siphons and also in the preparation of this paper. One of the many activities at the time of his death was experimental work in checking the control of breeding with the operation of the Morvant siphon. Mr. Shannon intended that a full review of these experiments would be prepared by him and appear in this paper. The authors feel strongly that his name should be associated with the work and acknowledge with gratitude his assistance and encouragement, together with that of the International Health Division of The Rockefeller Foundation.

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MEDICAL RESEARCH IN MALARIOLOGY IN THE FIRST POSTWAR YEAR, 1945-1946

Received for publication 5 November 1946

REPORT OF THE COMMITTEE ON MEDICAL RESEARCH*

The acceleration of malaria research during the war has resulted in significant advances in prevention and treatment, among which the following are outstanding:

(1) Demonstration of relationship between blood concentration and antimalarial activity of drugs (Brodie and Udenfriend, 1943; Shannon et al., 1944).

(2) Improvement in use of atabrine in treatment and suppression (Fairley, 1945; Joint Report, etc., 1946; S.G.O. Circular Letter, 1943; Shannon and Earle, 1945).

(3) Discovery of new and better antimalarial drugs, such as chloroquine (Board for Coordination of Malarial Studies, 1946; Most et al., 1946) and paludrine (Australian Army Research Unit, 1946; London Correspondent, J. A. M. A., 1945; Memorial, 1945).

(4) Employment of DDT in control of malaria-transmitting mosquitoes (Bishopp, 1946; Knipling, 1945; Knowles, 1945).

There has been an increase in studies on the exo-erythrocytic stages of plasmodia (Huff and Coulston, 1944; Hawking, 1945) and on the use of sero-diagnostic tests (Dulaney and Watson 1945). The problems of clinical relapse and the importation of malaria into new areas by returning troops have also attracted attention (Russell, 1945; Young et al., 1945).

With the end of the war, financial support of malaria research by governmental agencies, and international coordination of effort and exchange of information might be expected to diminish. The Committee on Medical Research has undertaken to survey the status of medical research in malariology during the first postwar year, and to note present trends as indicated by current or projected programs of various laboratories. Questionnaires have been sent to more than 100 institutions or individuals in the United States and abroad, and the information received is presented in this report. Since the Committee is concerned with medical research in malariology, programs dealing chiefly with entomology, engineering, epidemiology and the like, do not come within the scope of this study.

REPORTS FROM LABORATORIES IN THE UNITED STATES

Thirty-one laboratories in the United States have indicated that they are currently conducting research in medical malariology (Table 1). Analysis of data submitted shows the following points of interest:

(1) *Location:* All but one of the 30 laboratories are located in the eastern half of the country, the farthest west being at Galveston, except for the one in California. Only 11 are in the southeastern states, which have always constituted the chief region of malaria endemicity.

* Members of the Committee on Medical Research: Harry Beckman, Marquette University; Sterling Brackett, American Cyanamid Co.; David P. Earle, Jr., New York University; Victor H. Haas, U. S. Public Health Service, Chairman.

(2) *Type of institution:* The laboratories are operated by various types of institutions as follows:

Universities	17
Government (federal and others)	6
Pharmaceutical industries	5
Private foundations	3

TABLE 1

Summary of information reported by laboratories engaged in medical research in malariology in the United States, 1945-1946

LABORATORY	LINES OF INVESTIGATION	PLASMODIA USED	MOSQUITOES USED
American Cyanamid Co., Stamford, Conn. Sterling Brackett	Drug testing Parasite physiology	<i>gallinaceum</i> (s)	<i>A. egypti</i>
California Institute of Technology, Pasadena, Cal. J. B. Koepfli	Synthesis of compounds for antimalarials. Testing of drugs done elsewhere.		
Chicago, University of, Chicago, Ill. N. H. Taliaferro Clay G. Huff Alf Alving	Cellular and humoral fac- tors in immunity. Role of immunity in Chemotherapy Life cycle of parasite in vertebrate host. Plasmodia-mosquito rela- tionships. Effect of drugs on pre- erythrocytic stages.	<i>gallinaceum</i> (b.s.) <i>lophurae</i> (b.s.) <i>cynomolgi</i> (b.s.) <i>relictum</i> (b.s.) <i>catheimerium</i> (b.s.) <i>vivax</i> (b.s.) <i>elongatum</i> (b) <i>circumflexum</i> (b)	<i>C. pipiens</i> <i>A. egypti</i> <i>A. quadrimacu- latus</i>
Christ Hospital, Cincinnati, Ohio H. Schmidt	Drug testing	<i>cynomolgi</i> (b)	
Columbia University, Col- lege of Physicians and Surgeons, New York, N. Y. Robert C. Elderfield	Synthesis of new anti- malarials and chemical studies of known drugs.		
Emory University, Atlanta, Georgia W. B. Redmond	Cultivation of plasmodia in vitro metabolic require- ments of plasmodia	<i>relictum</i> (b)	

(b) indicates that blood-induced infections are used; (s) indicates sporozoite infections.

* *P. gallinaceum* maintained by U. S. Public Health Service, q.v.

† Includes station at Milledgeville, Georgia (Don E. Eyles).

‡ Strains: St. Elizabeth, Chesson, Pait *vivax*. U.S.P.H.S., Trinidad *malariae*. McLendon, Santee-Cooper *falciparum*.

§ Laboratory facilities being arranged for *P. catheimerium*, *P. relictum*, and *P. gallinaceum*, and for sporozoite production in *Aedes egypti*, *Aedes vexans*, and *Culex pipiens*.

|| Furnished as needed by U.S.P.H.S. laboratory at Columbia, S. C., q.v.

TABLE 1—*Continued*

LABORATORY	LINES OF INVESTIGATION	PLASMODIA USED	MOSQUITOES USED
Harvard University, School of Medicine and Public Health, Boston, Mass. Q. M. Geiman	Cultivation of plasmodia in vitro. Nutritional requirements of plasmodia. Metabolism of plasmodia. Correlation of in vitro and in vivo results. Effects of hyperimmune sera	<i>knowlesi</i> (b) <i>lophurae</i> (b) <i>vivax</i> (b) <i>falciparum</i> (b)	
Lederle Laboratories, Pearl River, N. Y. Redginal Hewitt	Search for new anti-malarials	<i>lophurae</i> (b) <i>cathemerium</i> (b)	
Marquette University, School of Medicine, Milwaukee, Wisconsin Harry Beckman	Survival of plasmodia in blood of refractory hosts.	<i>cathemerium</i> (b.s.)	<i>C. pipiens</i>
Maryland, University of, College Park, Md. Nathan L. Drake	Preparation of antimalarial drugs. Testing done elsewhere.		
Michigan, University of, School of Public Health, Ann Arbor, Michigan Richard J. Porter	Drug testing Life cycle of plasmodia in vertebrate host. Viability of sporozoites in various media.	<i>gallinaceum</i> (b.s.) <i>cynomolgi</i> (b.s.)	<i>A. quadrimaculatus</i> <i>A. aegypti</i> <i>A. albopictus</i>
New York University, Goldwater Memorial Hospital, New York, N. Y. David P. Earle Robert Berliner	Drug testing Action of antimalarials by in vitro methods	<i>falciparum</i> (b.s.)	<i>A. quadrimaculatus</i>
New York Public Health Institute of, New York, N. Y. Jules Freund	Immunization by means of antigens in lanolin-like substance	<i>knowlesi</i> (b) <i>lophurae</i> (b)	
North Carolina, University of, School of Medicine, Chapel Hill, N. C. J. C. Andrews	Relationship between nutritional deficiencies and quinine metabolism	none	
Notre Dame, University of, Notre Dame, Indiana Kenneth N. Campbell	Preparation of antimalarial drugs. Testing done elsewhere.		

TABLE 1—Continued

LABORATORY	LINES OF INVESTIGATION	PLASMODIA USED	MOSQUITOES USED
Parke-Davis, & Co., Detroit, Michigan A. C. Bratton, Jr.	Synthesis of new antimalarials Drug testing	<i>gallinaceum</i> (b) <i>cathemerium</i> (b)	
Rockefeller Foundation, New York, N. Y. Wilbur G. Downs	Exo-erythrocytic stages in chicks, embryos, and tissue cultures. Metabolism of plasmodia. Dilution methods for sporozoite inoculation. Drug testing	<i>gallinaceum</i> (b s.) <i>cynomolgi</i> (b.s.)	<i>A. quadrimaculatus</i> <i>A. egypti</i>
Rutgers University, New Brunswick, N. J. Leslie A. Stauber	Serological diagnostic tests	<i>lophurae</i> (b) <i>cathemerium</i> (b)	
Squibb Institute, New Brunswick, N. J. Arthur P. Richardson	Drug testing Pharmacological studies	<i>lophurae</i> (b) <i>cathemerium</i> (b)	
Station for Malaria Research, Tallahassee, Florida Mark F. Boyd	Host - parasite relationships, utilizing patients on malaria therapy service	<i>vivax</i> (b.s.) <i>falciparum</i> (b.s.) <i>malariae</i> (b.s.)	<i>A. quadrimaculatus</i>
Syracuse, University of, Syracuse, New York Reginald D. Manwell	Work ceased during war is just beginning. Will include cultivation of avian plasmodia and studying antimalaria drugs. Also will attempt to discover vectors for little-known plasmodia.	Will use avian species	Will use various culicines
Tennessee, University of, College of Medicine, Memphis, Tennessee Henry Packer R. R. Overman T. S. Hill	Clinical drug testing. Pathological physiology of malaria in man and monkeys. Exo-erythrocytic stages in embryos and tissue cultures. Serological diagnostic methods. Parasitology in avian host.	<i>vivax</i> (b.s.) <i>falciparum</i> (b.s.) <i>knowlesi</i> (b) <i>gallinaceum</i> * (b.s.)	<i>A. quadrimaculatus</i> <i>A. egypti</i>
Tennessee Valley Authority, Wilson Dam, Alabama E. Harold Hinman	Epidemiological studies. Insect - parasite relationship (proposed)	none at present	<i>A. quadrimaculatus</i>

TABLE 1—*Continued*

LABORATORY	LINES OF INVESTIGATION	PLASMODIA USED	MOSQUITOES USED
Texas, University of, School of Medicine, Galveston, Texas Wendell Gingrich	Immunology, including super-infection. Diag- nostic tests. Mechanism of relapse Exoerythrocytic forms Effects of drugs	<i>calhemerium</i> (b.s.)	<i>C. quinquefascia- tus</i>
Tulane University, School of Medicine, New Or- leans, La. Albert Miller A. J. Walker	Fate of plasmodia in re- fractory mosquitoes. Morphology of <i>S. Pacific</i> <i>vivax</i> strains. Nature of paroxysm. Host preferences of mosquitoes Effects of drugs	<i>vivax</i> (b.s.) <i>malariae</i> (b.s.)	<i>A. quadrimacu- latus</i>
U. S. Army, Army Medical Center, Washington, D. C.	Program currently limited to preparation of teach- ing material.		
U. S. Navy, Nat'l Naval Medical Center, Be- thesda, Md. L. A. Terzian	Reaction of host to para- site: factors influencing development of infec- tion and immunity	<i>gallinaceum</i> (b) <i>lophurae</i> (b)	
U. S. Public Health Serv- ice, Columbia, S. C.† Martin D. Young	Parasitology in man and mosquitoes. Immunity in human malaria. Host - parasite rela- tions. Transmission by mosquitoes under vari- ous conditions.	<i>vivax</i> (b.s.)† <i>malariae</i> (b.s.) <i>falciparum</i> (b.s.)	<i>A. quadrimacu- latus</i> <i>A.m. freeborni</i> <i>A. albimanus</i> <i>A c. crucians</i>
U. S. Public Health Serv- ice, Memphis, Tennessee V. H. Haas	Artificial immunization (chicks and monkeys). Modification of parasite by physical means. Exo-erythrocytic stages in chicks and embryos. Search for exo-erythro- cytic stages in monkeys. Plasmodia in refractory animals.	<i>gallinaceum</i> (b.s.) <i>cynomolgi</i> (b.s.)	<i>A. quadrimacu- latus</i> <i>A. egypti</i>

TABLE 1—*Concluded*

LABORATORY	LINES OF INVESTIGATION	PLASMODIA USED	MOSQUITOES USED
Winthrop (Sterling-Winthrop Research Institute), Rensselaer, New York Dr. M. L. Tainter Dr. E. W. Dennis	Screening and evaluation of new antimalarials. Mechanisms of therapeutic activity Mechanisms of infection and immunity which may be affected by drugs. Search for more suitable hosts for experimental malaria.	<i>lophurae</i> (b)§	
U. S. Public Health Service, Washington (Nat'l. Institute of Health, Bethesda, Md.) G. R. Coatney W. C. Cooper	Drug testing. Biology of plasmodia. Aspects of immunity Course of infection Pathology (in chicks) Plasmodia-mosquito relationships.	<i>gallinaceum</i> (b.s.) <i>falciparum</i> <i>vivax</i>	<i>A.egypti</i>
Virginia, University of, University, Va. Robert E. Lutz	Preparation of antimalarial drugs. Testing done elsewhere		

There is overlapping from the standpoint of financial support, in that most of the university laboratories receive assistance from governmental, industrial or foundation grants. The federal grant-in-aid program, administered by the U. S. Public Health Service, contributes at present \$261,263 (annual basis) to the support of 16 of the laboratories dealt with in this report, exclusive of those referred to above as government-supported.¹ It is quite evident that Federal financial support is an extremely important factor in keeping up medical malariological research in this country at present.

(3) *Lines of investigation*: Some laboratories report only a single line of study, while others indicate as many as 5 or 6 fairly distinct fields of interest. In general, studies are being conducted along the following lines:

- (a) *Immunology*, including studies on cellular and humoral basis for immunity, demonstration of immunity following infection and superinfection, and attempts at active and passive immunization—9 laboratories, of which 3 report interest in sero-diagnostic tests, and 3 indicate attempts to vaccinate animals or humans against various plasmodia.
- (b) *Parasitology*, including cytology, studies on the life cycle of plasmodia in vertebrate and invertebrate hosts, parasite physiology and metabolism, and attempts to cultivate plasmodia in artificial media—fifteen laboratories,

¹ The Committee is indebted to Dr. G. R. Coatney, U.S.P.H.S. for information relative to the Federal grant-in-aid program.

of which 7 are engaged in studies on the exo-erythrocytic cycle, 5 are investigating cultivation in vitro, and 5 are concerned with factors affecting plasmodia-mosquito relationships. Nutritional requirements and metabolism of plasmodia are being investigated by 3 laboratories.

- (c) *Drug studies*, including synthesis and testing of potential new antimalarials—19 laboratories. This type of study constitutes the sole line of investigation in 11 laboratories.
- (d) *Other investigations*: One laboratory is studying survival of plasmodia in blood fractions of refractory animals; One is interested in plasmodia in non-vector mosquitoes: Two are studying viability of sporozoites in various suspending media; One is concerned with application of single-cell techniques to plasmodia. A detailed study of pathological physiology in the mammalian host is being carried out by one laboratory.
- (4) *Host and parasite material*: The various malarias are maintained as follows:

- (a) Human malarias

Plasmodium vivax—7 laboratories are conducting studies on this species, although only 5 actually maintain their own strains, all of which produce sporozoites in *Anopheles quadrimaculatus*.

P. falciparum—4 laboratories

P. malariae—3 laboratories

- (b) Simian malarias

P. cynomolgi—5 laboratories (4 produce sporozoites)

P. knowlesi—3 laboratories

- (c) Avian malarias

P. gallinaceum—7 laboratories maintain this species, of which 6 produce sporozoites in *Aedes aegypti*.

P. cathemerium—7 laboratories use this malaria, only 3 producing sporozoites, grown in either *Culex pipiens* or *C. quinquefasciatus*.

*Other avian malarias*¹—*P. lophurae* is utilized by 7 laboratories (only 1 produces sporozoites); *P. relictum* is employed in 2 laboratories; *P. elongatum* and *P. circumflexum* are carried in only one laboratory.

Insectaries are maintained at 13 laboratories,² the following species of mosquitoes being regularly produced by the number of laboratories indicated:

<i>Anopheles quadrimaculatus</i>	8
<i>Aedes aegypti</i>	6
<i>Culex pipiens</i> or <i>quinquefasciatus</i>	3
<i>Anopheles punctipennis</i>	1
<i>A. maculipennis freeborni</i>	1
<i>Aedes albopictus</i>	1

Chick embryos are being employed in malaria investigations at 3 laboratories.

REPORTS FROM OTHER COUNTRIES

The Committee did not request information from countries outside the United States until the late summer of 1946, after most of the reports from this country had

² One laboratory is planning work with avian malarias but has not as yet established any species. This laboratory will also maintain an insectary.

been studied. Thus there has not been time to pursue the survey of medical research in malariology abroad beyond the stages of preliminary inquiry. At the time of preparation of this report, the situation relative to information from abroad is as follows:

(1) Correspondence from India indicates that the following programs are either under way at present, or are shortly to begin:

- (a) Malaria Institute of India,³ Delhi: The effects of anti-malarial drugs, and problems of immunity and relapse are being studied in monkeys, using *P. knowlesi*, *cynomolgi*; and *inui*. Sporozoites of *P. cynomolgi* are produced in several anophelines. Avian malarias are also employed, the species being *P. gallinaceum* and *P. relictum*; sporozoites of each are produced in several species of *Aedes* and *Culex* respectively.
- (b) Central Research Institute, Kasauli: A special program, called the "Mammalian Malaria Enquiry", is devoted to determining whether or not an exo-erythrocytic cycle occurs in mammalian malaria. Studies are conducted with *P. cynomolgi* in rhesus monkeys, sporozoites being produced in *Anopheles annularis* and *subpictus*.
- (c) Department of Protozoology, School of Tropical Medicine, Calcutta: Immunological studies are directed toward differentiation of strains of plasmodia, determination of comparative immunity resulting from blood-induced and sporozoite malaria and investigation into the possibilities of protecting humans against malaria by utilization of simian plasmodia. The exo-erythrocytic cycle in mammalian malaria is being sought for, and drugs, both Indian made and imported, are being tested for activity against simian and avian malarias. Parasites maintained are: *P. vivax*, *falciparum*, *knowlesi*, and *gallinaceum*, all except *knowlesi* being available in sporozoite form.
- (d) A large scale field trial of paludrine is planned, to be carried out under direction of the Malaria Institute of India, probably in Baluchistan.

(2) Malaria research in the Netherlands Indies⁴ apparently came to a virtual standstill during the war, but resumption of a number of studies, mostly along epidemiological lines, is being planned.

(3) Correspondence from Dr. Frank Hawking, Medical Research Council, London, states that a new committee, called "The Malaria sub-committee of the Colonial Medical Research Committee" has replaced the wartime malaria committee of the Medical Research Council. Dr. Hawking states that the new committee, and British malariologists generally, would be glad to maintain liaison with American workers. There has not been time to follow up specific requests for information as to research programs in England prior to the preparation of this report.

Dr. Hawking's own studies, carried on at the National Institute for Medical Research, Hampstead, are at present concerned with the search for possible tissue

³ For information from India, the Committee is indebted to Major-General Gordon Covell, Lieut.-Colonel M. K. Afridi; Col. H. W. Mulligan, and to the Director, School of Tropical Medicine, Calcutta.

⁴ The Committee is indebted to Dr. N. H. Swellengrebel and Dr. W. G. Venhuis for this information.

forms of *P. cynomolgi* in rhesus monkeys. Sporozoites are produced in *A. quadrimaculatus*. Chemotherapeutic studies are carried out in *P. gallinaceum* infections, and it is planned later to extend to *P. elongatum* and *Haemoproteus columbiae* the tissue culture of exo-erythrocytic forms which has been successful in the case of *P. gallinaceum*, *P. relictum*, and *P. lophurae*.

Reports from other countries have not been obtained, but preliminary inquiries have been made. The Chief of the Office of International Health Relations of the U. S. Public Health Service, who is working with the International Health Organization, has expressed interest in the proposal of the Committee on Medical Research to promote interchange of information between American Malariologists and those in other nations, and it appears that the building up of close liaison between this Committee (and probably other Committees of the National Malaria Society) and the International Health Organization might serve as the most effective method for promoting continuation of exchange of information on an international scope.

SUMMARY AND CONCLUSIONS

In response to questionnaires sent out by the Committee on Medical Research, 31 laboratories in the United States have reported on their investigations. Some information on research in medical malariology abroad has also been obtained.

Studies currently under way in the various laboratories cover practically every conceivable aspect of the subject. The most popular single line of investigation appears to be the development and testing of new antimalarial drugs, but research on basic problems of parasitology and immunology is not being neglected.

No significant diminution in medical research in malariology consequent upon the end of the war has as yet become apparent, but it is extremely significant to note that the great majority of laboratories in the United States engaged in this field, are dependent to a considerable extent on Federal government financial support.

The importance of avian malarias in research is noteworthy. Reports from laboratories both in the United States and abroad, show the extent to which avian plasmodia are utilized. In the United States, 15 laboratories use avian parasites, while only 8 use human and 7 use simian malarias. Of all species, *P. gallinaceum* is the most useful as an experimental tool.

Almost half the laboratories in the United States maintain insectaries; reports from abroad are not adequate for assessing the extent to which foreign workers utilize sporozoite infections.

The response to requests for information lead the Committee on Medical Research to conclude that exchange of information relative to programs and progress, is welcomed by virtually all workers in the field, and that this Committee, and probably other Committees of the National Malaria Society, can do a great deal toward promoting continuation of such exchanges of information as were customary during the period of intensified research engendered by the war.

RESÚMEN Y CONCLUSIONES

En respuesta a los cuestionarios enviados por el Comité de Investigación Médica, 31 Laboratorios en los Estados Unidos han reportado a cerca de sus investigaciones.

También se ha obtenido alguna información en relación con trabajos de Malariología en otros países.

Los estudios corrientemente en marcha en los distintos Laboratorios cubren prácticamente todo aspecto concebible sobre el sujeto. La línea popular más interesante de la investigación parece ser el desarrollo y el test de nuevas drogas antimaláricas, pero la investigación de problemas básicos de parasitología é inmunología no ha sido descuidada.

Hasta el momento no ha sido aparente ninguna disminución en investigación médica en Malariología consecuencial al fin de la guerra, pero es extremadamente significativo notar que la gran mayoría de los Laboratorios en los Estados Unidos ocupados en éste campo son dependientes de una manera considerable en soporte financiero del Gobierno Federal.

La importancia de las Malaria aviares en investigación es digna de hacerse notar. Informe de los Laboratorios, ya en los Estados Unidos, ya en otros países, muestran la extensión con la cual se están utilizando los *Plasmodiums* aviarios. En los Estados Unidos 15 Laboratorios usan parásitos aviarios, mientras que únicamente 8 usan parásitos humanos y 7 usan parásitos de simios. De todas las especies el *P. gallinaceum* es el más útil como una herramienta experimental.

Casi la mitad de los Laboratorios en los Estados Unidos mantienen insectarios; reportes de otros países no son adecuados para asegurar la extensión en la cual los trabajadores extranjeros utilicen las infecciones por esporozoítos.

La respuesta a las preguntas para información hacen concluir al Comité de Investigación Médica que el cambio de información relativa a programas y progresos, es bien recibido virtualmente por todos los trabajadores en el campo y que éste Comité y probablemente otros Comités de la Sociedad Nacional de Malaria, pueden hacer mucho hacia promover la continuación de tales intercambios de informaciones como se hacía por costumbre durante el período de investigación intensificada engendrada por la guerra.

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⁵ Work on antimalarials carried on under OSRD contracts is to be published in a 2 volume monograph entitled "A Survey of Antimalarial Drugs, 1941-1945." To be published by Edwards Brothers, Ann Arbor, Mich.

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BOOK REVIEW

"Practical Malariology," prepared under the auspices of the Division of Medical Sciences of the National Research Council. By PAUL F. RUSSELL, M.D., LUTHER S. WEST, PH.D., AND REGINALD D. MANWELL, Sc.D. 238 ill., 8 in color. 684 + XIX pp. W. B. Saunders Co., Philadelphia and London. 1946. \$8.00.

As its title conveys, this book is aimed to present practical observations, information and advice concerning malaria and its control. Such a caption promises many things to many people but, to a remarkable degree, the authors have delivered the goods.

Thus, the medical historian will find early in the volume a brief but informative resumé of important developments concerning malaria; frequent references of historical significance are made throughout the remainder of the book. The protozoologist will note an up-to-date summary of the nomenclature, taxonomy, life cycles, morphology and physiology of the malaria parasites of man and of closely-related forms in lower animals. Much of this information—especially the colored illustrations—will be of assistance to medical laboratorians engaged in the laboratory diagnosis of malaria. Both groups will be interested in the chapter on techniques used in the laboratory and in the field. Formulas and directions for preparing stains and culture media are included as well as protocols for serologic tests.

Entomologists and others concerned with anophelines will find the 220 pages devoted to mosquitoes (Section II and the "Keys to World Anophelines" in the Appendix) of special value. These provide very adequate treatment of the morphology, taxonomy, life cycles, and bionomics of mosquitoes; the global distribution of malaria vectors and of anopheline species by political sub-divisions of continents; field techniques, including procedures for making serviceable sketch maps for survey purposes; entomologic laboratory techniques, among which are featured methods for dissecting adult mosquitoes to reveal evidence of malaria parasitism and for precipitin-testing mosquito stomach contents to discover host preferences; and the identification of anopheline larvae and adults.

The next portion of the book deals primarily with the clinical aspects of malaria. It includes a discussion of the reactions to malaria parasites which constitute the pathology and symptomology of the disease; its clinical recognition and differential diagnosis, the normal and unusual courses of the infection, pernicious malaria, prognosis and sequelae. Treatment with quinine, plasmodin and quinacrine is considered in some detail; with the newer antimalarials, more sketchily due, presumably, to restrictions on publication when this book was written. The patterns of host-parasite relations and of clinical behavior of the different types and strains of malaria after primary attacks are reviewed. A separate chapter deals with the clinical aspects of blackwater fever.

Most of the remainder of the book is for the epidemiologist and the sanitarian. The interrelationships of biological, climatological, and other factors concerned in the transmission of malaria are well discussed. One chapter is devoted to an explanation of malaria epidemicity; another to malariometry and related survey procedures. A classification of "Measures of Malaria Prophylaxis and Malaria Control" precedes an orderly exposition of antimalaria measures by means of (1) the control of parasites in man, the feasibility of which with available drugs and biologicals under civilian conditions is dismissed; (2) larvicides, which are divided into four groups—oils, Paris green, DDT and miscellaneous; (3) drainage and filling of anopheline breeding places; (4) miscellaneous antilarval measures including naturalistic, mechanical, and water-management methods; and (5) the control of adult mosquitoes by naturalistic, mechanical, and chemical means. The "Prophylaxis and Control" section is especially comprehensive and well presented.

There is little that can be said in serious criticism of this fine book. It is well printed and bountifully illustrated. The authors meticulously define the sense in which they use words whose meanings might be obscure or subject to varied inferences. Some of the specific designations of anophelines as employed in this volume will undoubtedly shift with time. Perhaps the most serious shortcoming is the paucity of references to malaria literature. In spite of the authors' statement that their work is not intended to be bibliographic, that will not prevent its being quoted by persons unacquainted with original malaria literature as authority for statements in it not credited to someone else.

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Fourth International Congresses *on Tropical Medicine and Malaria* *Meet, May 10-18, 1948 at Washington, D. C.*

THE FOURTH INTERNATIONAL CONGRESSES ON TROPICAL MEDICINE AND MALARIA will meet in Washington from May 10 to 18, 1948, under the sponsorship of the Department of State with the cooperation of five government agencies and fifteen scientific societies. Over sixty governments have been invited by the Department of State to send official delegations. The Organizing Committee in charge of program and arrangements is made up primarily of representatives of the sponsoring bodies and has as its officers Dr. Thomas Parran, Chairman, Dr. George K. Strode, and Mr. Clarke L. Willard, Vice Chairmen; Dr. Rolla E. Dyer, Program Director; Dr. Wilbur A. Sawyer, Executive Secretary; and Mr. William L. Breese, Secretary. A comprehensive program has been prepared with emphasis on the great advances in tropical medicine since the previous joint meeting of the two international congresses on Tropical Medicine and on Malaria in Amsterdam in 1938.

There will be twelve scientific sections covering the following fields: research and teaching institutes, tropical climatology and physiology, bacterial and spirochetal diseases, virus and rickettsial diseases, malaria, helminthic diseases, protozoan diseases, nutritional diseases of the tropics, tropical dermatology and mycology, tropical veterinary medicine, public health, and medical and veterinary entomology. Visits will be made to the National Institute of Health in Bethesda, to the laboratories of the Department of Agriculture in Beltsville, and to other institutions and laboratories in and around Washington. Two evening sessions will be devoted to the recognition of great achievements in scientific medicine in the tropical field. One will commemorate the fiftieth anniversary of the discovery by Ronald Ross of the way in which malaria is transmitted, and the other will commemorate the demonstration by Walter Reed of the mosquito transmission of yellow fever. The delegates and members will be brought together in several social events.

In addition to the Official and Institutional Delegates, there will be enrollment of physicians, scientists, and other professional persons with qualifications and interest in the field of tropical medicine as members of the Congresses. Students, non-professional persons, and members of the families of professional members, may enroll as associates, without the right to participate in discussion or to vote. Those interested should write to the Executive Secretary, Fourth International Congresses on Tropical Medicine and Malaria, Department of State, Washington 25, D. C., U. S. A., for the Preliminary Announcement and the Advance Registration and Hotel Reservation form.

